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3032 ① plant respiration
② plant physiology
③ respiring system
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⑥ " quotient
⑦ respirable materials
⑧ glycolysis ⑩ oxidation
⑨ oxygen effects

PLANT RESPIRATION

BY

W. O. JAMES

M.A., F.R.S.

READER IN BOTANY, OXFORD

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I

THE RESPIRING SYSTEM

THE word respiration has been a part of the English language at least since the early fifteenth century. It was borrowed from Ciceronian Latin, and has naturally altered and developed its meanings during such long use. It is, indeed, a chameleon term that still changes its complexion against the varying backgrounds of different branches of biology. Although some of its meanings have become obsolete, several are still in use and liable to be met with in biological writings. It is necessary, therefore, to give them some passing attention and to decide our own usage.

The primary meaning of respiration is the action of breathing, the inspiration and expiration of a gas mixture, commonly air. This is the usual dictionary definition and the everyday use of the word. It is the sense in which Pepys entered it in his Diary[1] and which is still often intended by writers on human and animal physiology. A mass movement of gases by mechanical means is contemplated, and the mere exchange of gases by their own diffusion falls short of the intention. It follows that no plants can respire in this, the primary, sense of the word; as was early realized. Dutrochet, in 1837, defined the respiration of plants as the entry of oxygen into their air-spaces and its subsequent assimilation. He pointed out the similarity to the respiration of insects, and added that the green parts of plants were at an advantage because they generated oxygen within their air-spaces during daylight.

As the study of respiration advanced, it was realized that the obvious exchange of gases was only the most superficial aspect of respiration. The intake of oxygen was recognized as the antecedent of more significant internal changes within the tissues. In complex animals, which were the first organisms to be studied, the function of the breathing organs was recognized to be that they brought air into intimate contact with the blood. For a time, at least, this was regarded as the focus of the process. Huxley (1888) wrote that in the pulmonary capillaries 'an exchange very readily takes place between the blood and the air; the latter gaining moisture and carbonic acid, and losing oxygen. This is the essential step in respiration.' Even with this emphasis, the term is still inapplicable to plants, since the gas exchange that takes place in them is directly between air and living cells, not between air and an oxygen-carrying fluid. Even in the roots of swamp-inhabiting plants, where oxygen travels considerable distances within the organism, it is a movement by diffusion through intercellular spaces, not a mass movement mechanically accelerated. It was not until the application of respiration was widened to cover events taking place inside living cells that the term could be properly applied to the lowliest animal types and to plants in general.

---

[1] 22 Jan. 1665–6.

The secondary application of respiration is to the relation between cellular chemistry and oxygen, and this is the sense in which it is most frequently used by biochemists at the present time. This applies particularly to biochemists working with material of animal origin; and they are the great majority. Thus Meldrum described the subject of his book *Cellular Respiration* as 'the mechanisms by which the molecules oxidised are burned in the cells'. A popular textbook[1] says almost identically: 'The term *respiration* is applied to the process whereby oxygen is utilised for the combustion of food molecules.' Perhaps the most precise and closely-bound definition of respiration that has ever been offered is that of Szent-Györgyi (1938). 'If there is oxygen present, then succinic acid will be oxidised to fumaric acid, and malic to oxalo-acetic, and the two hydrogen atoms of the triose will be transferred on to this latter substance, which again transmits them to the Warburg-Keilin system, and so on to oxygen. This process we call respiration.' It shared with the preceding quotation the idea of cellular oxidation as synonymous with respiration.[2] Respiration in this secondary sense is sometimes spoken of as internal, or tissue, respiration and respiration in the primary sense of breathing, as external respiration.[3] The qualifying adjectives are usually omitted; they do not appear, for example, in the definitions just quoted, and it is rarely that there is anything but the context to show what sort of respiration is under discussion.

The secondary meaning can be applied to plants and was, in fact, so used by Sachs who referred to respiration as 'a very intense process of oxidation'.[4] But a difficulty arising from the existence of anaerobic fungi and bacteria was pointed out by Pfeffer.[5] 'The existence of such organisms is hardly in agreement with the old dogma "no life without respiration" unless we assume, as is here done, that respiration includes all metabolic processes that involve a liberation of energy.' He spoke of all respiration in the presence of oxygen as aerobic and all in its absence as anaerobic.

A tertiary meaning for respiration is thus introduced in which 'liberation of energy' replaces participation of oxygen as a criterion. It has been freely adopted by botanical writers, but less freely by other biologists. The physiologists and biochemists of animals often prefer to speak of changes in the absence of oxygen as anaerobic metabolism or glycolysis;[1] while microbiologists speak of fermentations.[2] Stevenson, it is true, decided to use respiration in the widest sense and announced in her monograph of bacterial metabolism that 'for practical purposes it can be used to denote any chemical reaction, aerobic or anaerobic, by which energy is liberated by the cell', even though she regarded anaerobic respiration as an almost self-contradictory phrase.[3] Anaerobic respiration and fermentation she treated as practically synonymous terms.

---

[1] Fearon, 1940.

[2] Cf. also Loeb, 1906, p. 13.

[3] Bayliss, 1920, p. 600, and numerous modern textbooks.

[4] 1887, p. 403.

[5] 1890, vol. i, p. 518.

In connexion with the higher plants, the use of 'anaerobic respiration' has become firmly established, and the great majority of plant physiologists, when they speak of respiration, are employing the word in its tertiary sense. By them respiration is indeed 'taken to include all the phenomena of dissimilation, the characteristics of which are the breaking down of complex substances into simpler ones with a consequent release of energy'.[4]

As a result of this emphasis on the energy relations of the process, it has been suggested that respiration should be confined to its primary meaning and that the term energesis be substituted for the tertiary.[5] Although a sufficient time has now elapsed, the suggestion has not achieved favour, and this is, on the whole, fortunate. It would be easy, as Meyerhof[6] pointed out, to assess respiratory events too exclusively on a purely energetic scale of values. The danger is greatest with plants, and recent writers have tended to stress this point.[7] The energy utilized by plants is not wholly derived from respiration; and, conversely, very little of the energy transformed during their adult respiration can be shown to have any functional significance (p. 242). Even the supply of chemical energy is not a sole prerogative of respiration unless we propose to include the oxidations of chemosynthesis within its scope. The tertiary definition of Stevenson mentions no specific class of substrate and so allows this; but the most usual botanical ones do not. The distinction is, indeed, very slender, and depends upon the supposition that the energy concerned in chemosynthesis is fixed in carbohydrates by processes analogous to those of photosynthesis; an assumption that is at present quite unproven. In practice, the term chemosynthesis is usually applied only when it is a question of the oxidation of simple inorganic substrates of external origin. That its energy transformations are linked to synthesis in general is no distinction from (tertiary) respiration, since the main demonstrable utilization of respiratory energy by plants also lies in forwarding the syntheses of growth.

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[1] See Baldwin, 1948; Bell, 1952.

[2] Gale, 1947.

[3] 3rd ed., pp. 12 and 44.

[4] Stiles and Leach, 1952, p. 1; cf. Wohl and James, 1942.

[5] Barnes, 1905.

[6] 1924, p. 86.

[7] Wohl and James, 1942; Briggs, 1943.

Even with more or less artificial exclusion of chemosynthesis, the modern notion of plant respiration becomes a very wide and generalized and even a fluctuating conception. A more restricted meaning is scarcely possible if only because there has never been, so to speak, a standard respiration. Respiration has evolved just as much as any other plant attribute, and has the variations and the indefinite boundaries so refractory to exact classification and nomenclature that are inevitably associated with evolutionary development. Without attempting to deny their importance or to lay exclusive stress either upon the transformation of energy or the utilization of oxygen, we shall take the term plant respiration to cover all those material changes undergone by complex cellular substances, themselves the product of primary syntheses. In the aggregate these changes lead to a diminution of free energy, which appears to have its importance because it is an invariable accompaniment of active life; but the immense variety of intermediate substances that arise may be of equal importance as starting-points of vital syntheses. Taken in this way, plant respiration proves to be a wider term than catabolism since it will be shown to include some inseparable anabolic syntheses. It is wider than the biochemists' (secondary) respiration because it includes stages that are not oxidations and that can proceed in the total absence of oxygen. It excludes fermentation only so far as fermentation has been appropriated by custom to the varied anaerobic dissimilations of micro-organisms. There is no natural reason for not considering the anaerobic respiration of higher plants as just the kind of fermentation of which that particular group of plants is capable. If a separation is attempted on the grounds that micro-organisms ferment external substances and higher plants anaerobically respire cell reserves, a new term will be needed for bacterial respiration, since that also depends on substrates coming from outside the cell. Nevertheless, it may be inexpedient to attempt to replace the term anaerobic respiration by fermentation in reference to higher plants, both because the group distinction is convenient, and because the term fermentation has already been used for plant processes, such as 'tea fermentation', that have turned out to be aerobic autolyses.

'No life without respiration'

There is general agreement that respiration is an invariable and essential attribute of living matter. This was upheld long before the real nature of respiration was apprehended, even vaguely. Crooke,[1] writing of the human body in 1615, remarked 'Life cannot be maintained without respiration', and fifty years later Hooke[1] was demonstrating to an early meeting of the Royal Society that life could be kept in a ribless dog by blowing air into the lungs. The application of the doctrine to lower animals and plants is more recent because the serious physiological study of such organisms was later in developing. Nevertheless, as early as 1679, Malpighi[2] observed that seeds require a supply of air for germination, and the connexion with respiration is—now, at least—obvious. Sachs, standing upon the threshold of modern plant physiology in a doorway that he himself did so much to open, remarked with characteristic emphasis '. . . the respiratory process is the first and most fundamental expression of the vital processes in protoplasm'. Later writers of weight have vied with one another in reiteration. 'Of all protoplasmic functions, the one which is, by tradition, most closely linked with our conception of vitality is the function for which the name of respiration has been accepted.'[3]

---

[1] *Oxford English Dictionary.*

According to Pfeffer, 'it has been observed, indeed, that isolated fragments of protoplasm continue to respire as long as they remain living'. The only difficulty associated with this dictum arises in deciding when is a thing living. At what stage of fragmentation, or under what adversity of conditions, does it cease to live, and does respiration cease at the identical point? That life might survive the suppression of respiration was at one time certainly believed. There were the 'livelie creatures shut up in the hard stones and living there without respiration or breathing as frogs, todes etc.'[4] The question is complicated by the idea that in such circumstances life itself might cease and afterwards be resumed upon the return of favourable conditions. In 1861 a committee of the Société de Biologie de Paris, under the presidency of Broca, reported itself satisfied that the inert bodies of primitive animals might for a time show no characteristics of life and yet revive. This might occur after 82 days in a dry vacuum or 30 minutes at 100° C.[5] The impossibility of demonstrating perceptible change and the difficulty in supposing any to have occurred were taken as showing that life was suspended, even though its normal manifestations were evident later.

On the botanical side, interest centres in the dormant seed, the frequent symbol of resurrection. The notion that all change might come temporarily to an absolute standstill was supported by Claude Bernard. He wrote:[6]

> It may be said that the life latent in seeds is purely potential; it exists ready to manifest itself if given suitable external conditions, but it does not show

---

[1] Hooke, 1667.

[2] See Stiles and Leach, 1952, p. 2.

[3] Blackman and Parija, 1928; see also Stiles and Leach, 1952, p. 2; Thomas, 1947, p. 3.

[4] Harrison, 1577, quoted from *Oxford English Dictionary*.

[5] Broca, 1861.

[6] Bernard, 1878, p. 69.

itself at all if such conditions are lacking. The seed has within itself everything needful for life, but it does not live because it lacks the necessary physico-chemical conditions. It would be wrong to suppose that in this instance the seed shows a life so attenuated that its signs escape observation by the very extent of their reduction. This is not true either in theory or in fact.

Bernard was impressed by the longevity of seeds, by the fact that they might retain their viability through centuries, even, of dormancy. 'These seeds', he concluded, 'have rested throughout so long a period as inert as if they had been really dead.' He supposed that were it otherwise, however slow the vital processes, they must over such long periods have caused enough change to become detectable.

The most astonishing things have been affirmed and believed about seed survival. The oldest known 'seeds' come from the tombs of the Pharaohs in which barley, wheat, and rye were placed to nourish the Ka or ghostly double of the deceased. Tourists of the mid-nineteenth century and later brought home from Egypt 'mummy wheat' capable of germination, and travellers' tales of these occurrences still circulated with the port in common rooms during the nineteen-thirties. Serious investigation of them was undertaken by Maspero, Director of the Museum at Cairo, and his findings were reported in 1901. He stated succinctly that the germination experiments fell into two series; those made with seed bought from fellahs as coming from the tombs, and those made with seed taken from the tomb by the germinater in person or handed to him by a reputable investigator. Seeds of the first class almost always sprouted; but seed of the second class had never come up to his knowledge. The conclusion, he thought, was readily drawn. At about the same time Gain[1] examined wheat from tombs of the ninth dynasty, *c.* 2400 B.C., and found that the embryos had turned reddish brown, suggesting a slow oxidation, and that the tissues fell apart and crumbled to powder. Subsequent investigation has, in fact, shown that cereal grains are short-lived, and under normal conditions of storage rarely retain their viability for more than 15 years.[2] Although some seeds may last longer, the oldest at present known to have germinated came from a hard-coated *Nelumbium* in the Sloane collection, and was only about 237 years old.[3] Really long periods do allow enough change for complete loss of viability, and, no doubt, more refined observation would reveal smaller changes in much shorter times. Hard coats,[4] low moisture content down to about 2 or 3 per cent., scarcity of oxygen, and low temperature tend to a long storage life. These are also conditions that might be expected to retard respiration. There appears to be no lower limit to the removal of oxygen, providing the seed's moisture content is low; and dry seeds have been subjected to the temperatures of liquid air[1] and liquid helium (1·35–4·2° K.)[2] without loss of vitality. Excessive removal of water, on the other hand, destroys them finally, and some will not stand desiccation at all. The statement sometimes made that seeds when quite dry have no respiration is equivalent only to saying it of those that are dead.

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[1] Gain, 1900, 1901.

[2] Sifton, 1920 (wheat), see Lehman and Aichele, 1931, p. 196, for summary of other cereals.

[3] *Nature*, **149**, 653, 1942; *Gard. Chron.* iii. 234, 1942.

[4] Becquerel, 1907, 1934; Turner, 1933; Crocker, 1948.

There is thus abundant evidence that seeds may remain alive, living very slowly, for long but not indefinite periods. In spite of Bernard's belief to the contrary, some changes, however slow, must be supposed to continue during dormancy. Van Tieghem[3] after experimenting with dormant peas, relatively thin-walled leguminous seeds, in atmospheres of air and carbon dioxide, came to the conclusion, 'Respiration thus continues during dormancy and, if it cannot do so, the organism dies.' Although van Tieghem's experimental arrangements might not stand up to modern criticism, they were soon supported by more extensive results. Under conditions that are normal or that approach normality, many dormant seeds may be shown to give off carbon dioxide[4] and consumption of oxygen has been demonstrated also.[5] Seeds in contact with air normally have a moisture content within the range 10–20 per cent. If the water content is reduced, carbon dioxide output, in contrast with viability, is reduced also.[6] In the detailed results of Bailey and Gurjar, reduction of carbon dioxide output is very great between 17·07 and 14·78 per cent. moisture; but very slight between 14·78 and 12·50 per cent. There is no indication that, with further drying within the viably safe limits, respiration would necessarily drop to zero. Earlier workers had claimed to show that artificially dried seed, although it retained its capacity for subsequent germination, showed no gas exchange whatever.[7] White (1909) was able to demonstrate the emission of traces of carbon dioxide from air-dry grain of oats, barley, rye, wheat, maize, and Italian millet. After 8 days drying at 45° C., only the maize was still demonstrably 'respiring'. This, of course, is only equivalent to saying that the others were no longer producing enough carbon dioxide to be detected by the methods she employed; and it has been calculated[8] that the carbon dioxide productions since observed in cereals would not have been detectable by her technique.

---

[1] Lipman and Lewis, 1934.

[2] Lipman, 1936.

[3] Van Tieghem, 1891, p. 938.

[4] See, for example, Maquenne, 1900; Kolkwitz, 1901; Duvel, 1904; Becquerel, 1907; Jauerka, 1912; Crocker and Harrington, 1918; Smith and Gane, 1934. Older literature is reviewed by Becquerel (1907) and Pfeffer (*Physiology of Plants*, ii. 254).

[5] Bakke and Noecker, 1933; Merry and Goddard, 1941.

[6] Kolkwitz, 1901 (barley); Bailey and Gurjar, 1918 (wheat).

[7] Romanes, 1893; Giglioli, 1895; Jodin, 1899.

[8] Merry and Goddard, 1941.

From our present angle van Tieghem's conclusion is perhaps the crux of the matter. To the extent to which it respires the protoplasm remains alive. Up to the present, no conditions have been devised that clearly separate the respiring from the living state. So far as survival under conditions of cold and desiccation, extreme enough to preclude chemical change, is concerned the comment of F. F. Blackman (1908*b*) remains the last word. 'It may well be that there will not be enough CO_2 produced to be detectable in ten years; but who shall say that change has *ceased*? Our methods of analysis which demand a large aggregate of molecules for any demonstration, are incapable of settling this philosophical question.'

One point remains to be considered, viz. whether the gas exchanges demonstrated in normally dormant seeds fall within the definition of respiration. Blackman thought it rather doubtful, and pointed to an experiment of Becquerel's in which isolated testas, which contain only a very small proportion of the living tissues, gave off as much carbon dioxide as the whole seeds before stripping. Further, the gas exchange of intact dry seeds was found 'unlike respiration' to be accelerated by light and especially by blue light. The results of Bailey and Gurjar already mentioned might also be explained on the hypothesis that true respiration ceases at about 12 to 15 per cent. moisture content, and that only a very slow gas exchange of different type, and more or less independent of moisture, persists below it. Blackman thought that the slow gas exchanges might indicate a purely photochemical oxidation of seed materials, which would in time destroy the organization necessary to maintain viability. Viability is lost without any considerable depletion of seed reserves such as later occurs in the rapid respiration of germination, and the gas-exchange ratio (CO_2/O_2) of dormant seeds is much lower than the normal.[1] It is therefore reasonable to suppose that the slow changes in the dormant grain differ in kind as well as in rate from those of the seedling. It would be very difficult to decide whether they are radically different from respiration as usually understood, not only because their slowness makes them hard to investigate accurately, but also because of the difficulties in defining respiration.

So far as dormant seeds are concerned, we might therefore still be upon the horns of the old dilemma; either they live without respiration, with extreme sluggishness no doubt, but still live; or, as Claude Bernard thought, they cease to live and come alive again. The first alternative cannot be strictly reconciled with the dictum 'no life without respiration'. No objection can, on the other hand, be raised if we are prepared to relax a little of its rigour and say that active life cannot exist without active respiration.

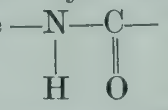
---

[1] Harrington, 1923; James and James, 1940.

Catalytic protoplasm

The constituents of living protoplasm may be divided into two categories, the respiring and the respirable. The former comprises the catalytic machinery of respiration, a small amount of which is capable of promoting a large amount of metabolic change, and the latter the plastic substrates that are transformed. The division is not absolute and, since the catalytic protoplasm may grow as respiration proceeds, it is evident that some plastic material must pass from the one category into the other. Protoplasm is not simply catalytic; but autocatalytic as well.[1]

The term protoplasm might be defined to exclude all substrates; but such a residue would not exhibit the properties of life which are characteristic only of the complete system. Modern studies of the molecular structure of proteins throw some light on the probable nature of respiring protoplasm. The proteins so far studied fall into two rather sharply defined categories, the more or less insoluble, fibrous proteins and the soluble, globular proteins. The terms fibrous and globular may be taken as indicating the shape of the molecules, the former class having long 'chains', threads, or fibres; and the latter globules that are perhaps built up of chains thrown into numerous orderly folds. Examples of fibrous proteins are silk fibroin and the keratin of mammalian hair; both highly insoluble. Typical globular proteins are seed reserves such as zein from maize, edestin from hemp, and excelsin from spruce. Unlike the fibrous proteins, these have determinable molecular weights, all of which appear to be multiples of 17600[2] (zein = 17600 × 2; edestin and excelsin = 17600 × 16). Although they are so large, their compact shape allows the molecules to become regularly arranged in crystal lattices, and many globulins have now been had in crystalline form. The reserve protein of castor-oil seed forms crystals in the living cells that were well known long before crystalline proteins were obtained artificially.

The structure of the protein molecule is most readily revealed in those that are fibrous. The well-known peptide linkage  $-\underset{\substack{| \\ \mathrm{H}}}{\mathrm{N}}-\underset{\substack{\| \\ \mathrm{O}}}{\mathrm{C}}-$ leads to the formation of chains of more or less indeterminate length. These chains may contract and fold in varying degrees but, when stretched as in a silk thread under tension, the chains straighten out, and, becoming crystalline, reveal regularities of arrangement in X-ray diffraction patterns. In silk fibroin there is a regular repetition, or fibre period, of 6·95 Å. Side chains occur alternately on each side of the main valency chain, and the distance between side chains is 3·5 Å. The corresponding interval in β-keratin has been estimated at 3·38 Å.[1] The structure of the chain is therefore of the general type,

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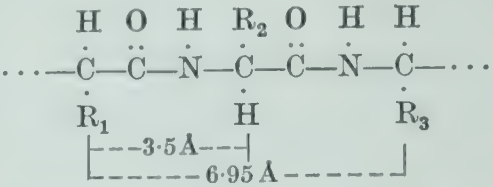
[1] Cf. F. F. Blackman, 1908*a*.

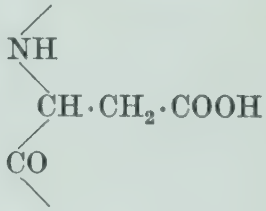
[2] Svedburg, 1931, 1938. See also Frey-Wyssling, 1948.



$$\cdots-\overset{H}{\underset{R_1}{C}}-\overset{O}{C}-\overset{H}{N}-\overset{R_2}{\underset{H}{C}}-\overset{O}{C}-\overset{H}{N}-\overset{H}{\underset{R_3}{C}}-\cdots$$

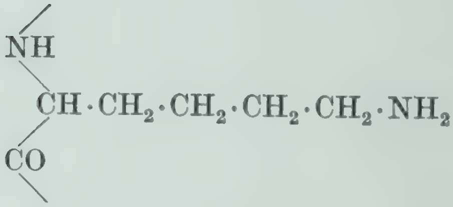
The group $>CH \cdot NH \cdot CO-$ is common to all amino-acids and is contributed by each to the main chain; the radicles R_1, R_2, R_3 ... vary with the amino-acid. The acid and basic properties of the carboxyl and α-amino groups are lost in the formation of the peptide linkages; and glycine, with a 'side chain' of a single H atom, can contribute little to the further properties of the protein. These depend on the side chains of the more complex amino-acids. Thus, the amphoteric behaviour of the proteins is brought about by the additional carboxyl groups of aspartic and glutamic acids and the additional basic groups of amino-acids, such as lysine and arginine. Examples of amino-acids forming side chains giving important properties to protein molecules are listed below:

Acidic



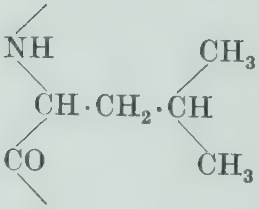
aspartic acid

Basic



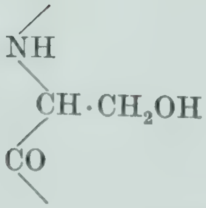
lysine

Fat-soluble



leucine

Water-soluble



serine

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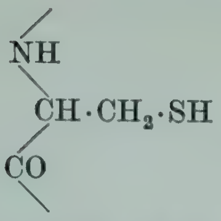
[1] Astbury, 1933.

S-containing



$$\begin{array}{l} \diagup \\ \mathrm{NH} \\ \diagdown \\ \quad \mathrm{CH}\cdot\mathrm{CH_2}\cdot\mathrm{SH} \\ \diagup \\ \mathrm{CO} \\ \diagdown \end{array}$$

cysteïne

In silk fibroin the amino-acids are nearly all glycine and alanine, with side chains —H and $—CH_3$ respectively. The valency chains can therefore lie regularly side by side, and so form a crystal lattice in much the same way as cellulose. Protoplasmic proteins have more than twenty different kinds of side chains of very different lengths and shapes, and have little chance of settling into a regular lattice; in other words they do not crystallize and remain permanently colloidal.

The side chains, according to their various natures, may enter into a variety of reactions. One most important result is that bridges may be built, linking one polypeptide chain with another. Thus, an acidic lateral on one chain may form a salt bridge with a basic group, or an ester linkage with an alcohol side chain (serine) on another. Oxidation of two thiol side chains of cysteïne may make a cystine sulphur bridge. The formation of such bridges probably accounts for the insolubility of the fibrous proteins and for their comparative firmness and resistance to hydrolysis.

The known physical properties of protoplasm, its ability to flow, combined with plasticity and a degree of elasticity, as well as its high water content, can be accounted for on the supposition that it consists fundamentally of long interweaving polypeptide chains widely spaced in water and linked here and there through groups of side chains. To account for its great fluidity it must be supposed that, at least during flow, the junctions are continually breaking down and continually being renewed. Protoplasm differs in this from any non-living gel.

Only a small proportion of the side chains are saturated in bridge formation. The great majority remain free to react with non-protein materials, which may thus become attached to the protein framework. Fats, for example, may become attached to lipophilic side chains and the lecithins, being themselves of dual nature, may become attached both to those that are lipophilic and those that are hydrophilic as well. Auxin is held very tenaciously, possibly by an ester linkage, on a cytoplasmic protein of spinach. It is not released by electrodialysis, or dilute alkalis in the cold; but only by prolonged alkali hydrolysis at 100° C.[1]

Enzymes are proteins, either with or without other essential groups attached. Their proteins, when isolated and purified, are of the globular type and many, some fifty or so, have now been crystallized. Reasons have been advanced for supposing that globular proteins are formed of chains folded regularly into more or less isodiametric complexes, and that there is no fundamental difference of structure between the globular and fibrous types. Protoplasm exhibits properties that are intermediate between the two extremes; it does not crystallize like the globulins, but neither is it so insoluble as the fibrillar kinds. It may contain proteins of both types, or even oscillate with varying degrees of folding between the two conditions.

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[1] Wildman and Bonner, 1947.

It is noteworthy that enzymes differ very much in the ease with which they may be separated from the cell structure. Most may be freed by simple extraction, i.e. dilution with water followed by mild physical methods of precipitation or adsorption. It would not appear that any strong chemical bonds such as peptide linkages are broken, and it would be difficult to suppose that such enzymes are joined to other enzymes or non-catalytic proteins, except by some of the weaker side-chain linkages. The extreme example is afforded by the exoenzymes that are secreted in free solution by insectivorous plants, the barley scutellum, and saprophytes generally. At the other extreme there are enzymes that are retained tenaciously by the cell structure, and which are only liberated after it has been drastically disorganized by autolysis, or by freezing which breaks down the system by dehydration. Many enzymes, of which plant dehydrogenases are notorious examples, become very much less stable after separation by such means. They would appear to be more firmly bound than the easily extractable and stabler hydrolases. Lactic dehydrogenase of barley is liberated by 12 hours of freezing at —12° C.; but after 24 hours is inactivated,[1] perhaps because its globular protein is itself denatured by rupture of its folded chains. Some few enzymes, have never been separated from cell detritus and are associated with specialized bodies; cytochrome oxidase, for example, is believed to occur almost wholly in mitochondria.[2]

Oxidizing enzymes also possess non-protein groupings that are essential to their action. These are of various kinds, and are attached to their proteins with varying firmness. Thus Coenzyme I (adenine-nicotinamide dinucleotide) sets up a variety of enzyme systems in association with different proteins; but is easily removed by simple dialysis against water from all of them. Diphosphothiamin, the coenzyme of carboxylase, is rapidly removed only by alkaline dialysis. It can be linked to its protein in yeast by a divalent metal,[3] probably magnesium in the living cell. As both metal and coenzyme are displaced

[1] Beevers, unpublished.

[2] Schneider, 1946, see also Lang, 1952; Robertson, Wilkins, and Weeks, 1951.

[3] Green *et al.*, 1941; Kubowitz and Lüttgens, 1941.

by dilute alkali, a salt linkage is presumably formed through the metal with an acid side chain. Copper, which forms the prosthetic group of polyphenol oxidase, is held much more firmly by a covalent instead of an electrovalent link to its protein, and cannot be wholly removed by prolonged dialysis, even against cyanide. The whole enzyme including the protein is, however, readily removed by simple crushing and water extraction.

It is thus possible to think of the catalytic machinery of protoplasm as a three-dimensional network, the threads of which are somewhat loosely held together, and which retain essential parts of the machinery more or less firmly fixed along their lengths. The centres of activity will not be rigidly separated by constant distances; but neither will they be free to disperse at random in the water-filled interstices of the net. Soluble substrates will be in a more favourable position in this respect and, except perhaps at the plasmatic boundaries, may be supposed able to diffuse freely until they impinge upon a catalytic centre; reactions will therefore be heterogeneous between more or less fixed enzymes and the relatively free substrates.

Organization resistance

Organized protoplasm behaves very differently from a mere collection of enzymes. It grows, and in doing so habitually brings about reactions extremely improbable in what Leathes (1926) termed 'the rough and tumble of ordinary liquid systems'. There is no reason to suppose that a substrate—say a sugar molecule—when dissolved in the water of the cytoplasm acquires properties beyond those it possesses in any other solution. Nevertheless, it undergoes protean transformations, none of which it would suffer uncatalysed, and some of which it would not perform even in the presence of unorganized enzymes. It is not true to say that none of the reaction sequences characteristic of living cells will persist if the cell organization is disrupted. The long and complex series of reactions leading from glucose to alcohol is catalysed by zymin in which the protoplasmic structure has been thoroughly disorganized by acetone and ether. Similarly, clear saps from frozen barley have been shown to produce pyruvic acid from sucrose and break it down further with release of carbon dioxide.[1] It is, however, significant that to bring this about adenylic acid had to be added to the solution. It is difficult to believe that these clear solutions possessed enough structure to ensure any fixed spatial relationships between the rather numerous enzymes, at least seven, concerned.

Having regard to the proved presence of both enzymes and substrates, the probable reactions that do not occur are perhaps as significant as

[1] James and Bunting, 1941; James, James, and Bunting, 1941.

the improbable ones that do. When the protoplasmic structure is wholly or partly destroyed, these may begin, or, if already proceeding slowly, be greatly accelerated. The classic example is the oxidation of polyphenols by oxidases leading to the formation of post-mortem colours. Less familiar is the destruction of tropane alkaloids that are stable in belladonna tissues until protoplasmic breakdown sets in, when they disappear rapidly. The term organization resistance was coined by F. F. Blackman to describe the plasmatic suppression of such reactions and, like others, he ascribed it to a spatial separation of enzymes and substrates, and thought it likely to be due to 'the adsorption or combination of one or both of the reactants by the stabilised components of the protoplasm'. Although this opinion was expressed before the recent studies of protein structure had been carried out, it harmonizes well with the views stated above. As another, possibly less important, factor separation by impermeable protoplasmic membranes was suggested. Belladonna alkaloids follow the general rule for alkaloids and accumulate in the vacuole, so it is not difficult to suppose that in the normal conditions of the cell they are separated by a membrane from the enzymes effecting degradation. The same may be true of the polyphenols, at least in large part. Another rather similar kind of separation is suggested by the occurrence of localized bodies, the plastids, within the general cytoplasm. Chloroplasts and amyloplasts are well known to be the only sites of starch formation, and recently it has been shown that phosphorylase, which catalyses one of the final stages in starch synthesis, is restricted to the same plastids.[1] Glucose-1-phosphate in the cytoplasmic water outside the plastids would be 'protected' from this type of reaction.

Spatial organization may be effective not only in suppressing reactions but also in promoting them. Glucose-1-phosphate, if localized outside plastids, would not be converted to starch and might be converted to hexose-1, 6-diphosphate at a phosphorylating centre instead. It will be shown in Chapter X that one of the most important features of respiration is the linking of endergonic and exergonic partial reactions. Such linkage is one of its most difficult features to reproduce *in vitro* in unorganized solutions, and may conceivably occur in the living system on account of the close spacing of the necessary reaction promoters.

If one adopts the picture of freely moving substrates reacting at more or less fixed centres of catalysis, it is evident that the arrangement of the centres may have far-reaching effects upon the sequence of reactions that develops. In the simplest possible case a substrate, A, may be supposed to react at a centre, a, to give a product, B, capable, in the presence of catalyst b_1, of further conversion to C, or in the presence of b_2, to D. If the molecules of b_1 are located near to those of a, and those of b_2 are on the average farther away, the diffusion gradient of B will give it a higher concentration at the b centres, and, other things being equal, a faster rate of reaction with them. The product C will therefore tend to be formed in excess of D, or even to its virtual exclusion. This state of affairs would obtain even in the complete absence of membranes impermeable to the reactants. Many of the respiratory reactions are a general property of cytoplasm and probably cannot be limited to special organelles. Distribution of catalysts as described above may therefore have much to do with their rates and occurrence. Although post-mortem respiration sequences may continue, as in zymin and barley saps, they differ from the normal process in rate, kind, and persistence.

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[1] Yin, 1948.

II

THE RATE OF RESPIRATION

RESPIRATION is a complex sequence of chemically reversible and irreversible reactions and, neglecting catalysts, may be represented:

$$A \underset{k_1}{\overset{k_{\mathrm{I}}}{\rightleftharpoons}} B \underset{k_2}{\overset{k_{\mathrm{II}}}{\rightleftharpoons}} \cdots (+\mathrm{O}_2) \quad \cdots \underset{k_y}{\overset{k_Y}{\rightleftharpoons}} Y \underset{k_z}{\overset{k_Z}{\rightleftharpoons}} Z.$$

So long as a steady state operates, the rate of the process may be measured without ambiguity by change of either reactant or product; but alterations of controlling factors may affect the velocity constants $k_{\mathrm{I}}\ k_{\mathrm{II}} \ldots k_1\ k_2$, &c., differently. Until a new steady state is established, rates of consumption and production will differ to the extent that the concentrations of intermediates are changed to establish the new equilibria. In practice, respiration rates are measured in terms of oxygen consumption or carbon dioxide production, or both, according to the particular end in view. Consumption of the ill-defined and variable substrate, A, is too inaccessible for practical use.

Of the required parameters, oxygen and carbon dioxide concentrations and time are capable of exact measurement; and the 'respiration rate' of a given plant or tissue can therefore be expressed with accuracy. Difficulty arises when it is desired to compare the respiration rates of two separate tissues or bodies, because of uncertainty as to the unit of respiring matter. We can measure a rate; but we cannot obtain from it a standard rate comparable to the velocity constant, k, of a catalysed chemical reaction with $v = kS^nE^x$. The unit of respiring matter does not contain a single enzyme, E, with characterizing properties, but a collection of enzymes any one of which may dominate the respiratory rate under varying circumstances. Such a 'unit' is not susceptible of experimental investigation and, even in theory, has no precision.

Respiration rates are therefore commonly referred to some arbitrary unit of weight, surface, or volume of tissue, and are then sometimes spoken of as respiratory activities or intensities.[1] The application of the adjective respiratory to all such terms is obviously conventional, and in need of *ad hoc* definition. Something would be gained if, instead, one adhered to more precise phrases such as 'gm. CO_2 per gm. fresh weight per hour'. The commonest bases of comparison follow, with a brief discussion of their uses and limitations. They include cell number, the individual organism or tissue, surface area, fresh weight, dry weight, nitrogen, protein, and selected protein fractions.

[1] See Stiles and Leach, 1952; Thomas, 1947.

UNITS OF RESPIRING MATERIAL

The cell. The first unit that suggests itself in the measurement of a vital process is the cell. In populations of free living unicells, the effects of external conditions, ageing, mutation, and so on may well be referred to the respiration per cell. Conversely, the growth of yeast cultures (i.e. the number of living cells present) has been estimated by measurement of the respiration under standard conditions.[1]

In uniform tissues containing cells of only one type, the individual cell might again be a satisfactory denominator for comparisons. Mature apples that have stopped forming new cells show a correlation between the number of cortex cells and rate of CO_2-production. The calculated rate per cell is not actually the same for different varieties, but varies by a factor of 2. The fastest rate observed at 12° C. was $6{\cdot}4\times10^{-8}$ mg. per hr. per cell (Allington Pippin), and the slowest $3{\cdot}2\times10^{-8}$ (Lord Derby). When respiration rates of different varieties were compared on a basis of equal weights of tissue, the fastest rates were associated with fast ripening, and slow rates with good keeping qualities; but comparison on a basis of equal cell numbers showed no similar regularity.[2] Even in such simple examples respiration is not strictly related to cell number, and there is no sudden increase of respiration at cell division. Gray (1931) contrived to have a culture of fertilized eggs of *Echinus esculentus* which divided in unison. Total oxygen consumption increased steadily from the 1-celled to the 8-celled stage without any jumps at the times of cleavage. The drift of respiration values depending on the ageing of cells is well known and has been extensively studied in apples (e.g. p. 70).[3] Cell number can only rarely be a useful basis for a comparison of respiration rates, and, on the practical side, it is far from easy to obtain a satisfactory estimate of the number of cells in a piece of tissue.

The organism. The individual organism is a natural unit that may often be the logical basis of comparison. It is possible to measure the CO_2-output of a single plant[4] over considerable periods and under varying circumstances. The results obtained will be individual to the experimental victim, usually dead when the experiment ends. If results are desired that may be considered characteristic of the variety or species, the use of samples is inevitable. The estimation of the mean rate for the species, under defined conditions, from that of an experimental sample is governed by the relation

$$S = \sigma/\sqrt{n},$$

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[1] See Slator, 1913.

[2] W. H. Smith, 1936*a*, 1937.

[3] Robertson and J. F. Turner, 1951.

[4] e.g. Stiles and Leach, 1933.

σ and S being the standard deviations of the species and sample means respectively, and n the number of individuals in the sample. For estimating the mean rate, a sample of n individuals is $\sqrt{n}$ times better than a singleton. From this point of view it is better to measure the respiration of a large number of small individuals than a small number of large ones, and seedlings are oftener compared on this basis than adult plants. Moreover, not only does the bulk of the adults prohibit the experimental handling of large numbers, but their variability increases with age, and, when variability rises, the number of individuals in a sample required to maintain the same value of S goes up with σ^2. For these reasons, unitary comparisons of multicellular adults is rarely practised.

The organ. Attempts to reduce sample size to manageable proportions have been made by selecting simple individuals or specific organs. Leaves have been a favourite choice in spite of their complex anatomy. Roots show a very pronounced respiratory gradient backwards from the apex (Machlis, 1944*b*), but useful comparisons can frequently be made on a basis of counting tips.

Surface area. In some circumstances, it is advantageous to express respiration rates in relation to unit surface of the tissue; especially with leaves when it is desired to use the data in connexion with measurements of photosynthesis. Thus Blackman and Matthaei (1905) found it convenient to express the respiration of sunflower and cherry-laurel leaves as gm. CO_2 per hr. per 50 sq. cm. It need hardly be remarked that the organ has two surfaces, and that the question may be complicated by their unequal permeabilities to oxygen.

Specific surface. It has sometimes been proposed to relate respiration rates of bulky tissues to the surface/volume ratio. This would be desirable if respiration were limited by the rate of entry of oxygen or escape of carbon dioxide; but such instances seem rare among plant tissues. They are discussed on pp. 142 et seq.

Fresh weight. Tissues that are not markedly dissimilar can be compared on the basis of their weight as gathered. This is obviously a rough practical criterion that has no general theoretical basis, and has marked shortcomings even with such apparently uniform material as the flesh of apples. Nevertheless, it has been used with success in a variety of respiratory investigations. Its ease of determination often makes it preferable to other criteria, where complex handling of the material leads to no corresponding gain. A special point in its favour in respiration experiments is that these are usually carried out in an atmosphere (often saturated) that is little subject to humidity changes, and there is no risk of large alterations of the tissue's water content. In many experiments such changes are, in this respect, immaterial since results can be referred to initial fresh weight. This is perhaps the most used basis in drift experiments.

Dry weight. Correction for variations of water content can be made by drying the tissue to constant weight at a standard temperature, usually 100° C. This standardizes the most obvious of the cell variables at the cost of destroying the system. Dry weight can, therefore, only be obtained by collateral sampling or as it exists at the end of the experiment. In spite of these disadvantages, dry weight is the most used standard of reference between two samples of tissue, and much useful information has been collected by comparisons based upon it. It is sometimes possible to correct for the grosser vagaries introduced by its use.[1] There is no standard reference weight in common use. Kidd, West, and Briggs in an ontogenetic study of respiration defined the *respiratory index* as mg. CO_2 per hr. per gm. dry weight, measured at 10° C., 21 per cent. oxygen, and in the presence of excess respirable material. The last proviso will be referred to again below. The index has not come into general use, but more recently an attempt has been made to standardize the quantity Q_{CO_2}, especially in connexion with manometric measurements. This is defined as cu.mm. (N.T.P.) CO_2 per hr. per mg. dry weight (Dixon, 1934). Q_{O_2} is the corresponding quantity for oxygen, often expressed with negative sign to indicate uptake.

Nitrogen and protein. The real objection to the foregoing methods of comparison by weight, number of cells, and so on is that they inevitably confuse two contributions to respiration rates which vary independently. The velocity of a catalysed reaction is a function of both reactant and catalyst concentrations, and can be expressed

$$v = k_1 S^m + k_2 S^n E^x.$$

In most biological reactions the velocity of the uncatalysed reaction, $k_1 S^m$ can be neglected; but in the second term of the equation the concentrations of both reactant and catalyst occur. In a respiring tissue, reactants are represented by plastic materials and catalysts by the catalytic protoplasm, as already elaborated on p. 9. Some mention has been made there of the difficulty of sharply separating plastic and enzymatic materials in practice, or even in theory. Attempts have nevertheless been made to do this, so that respiration might be referred to a unit based on the catalytic, as distinct from the total, material of the tissue. The respiratory index of Kidd, West, and Briggs (above) specifies an excess of respirable material so that respiration rate is controlled by the amount of catalytic protoplasm ($v = kE^x$). It does not, however, provide any means of measuring E. This had already been attempted by Palladin (1896). He started from the observation of

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[1] Cf. Kidd, West, and Briggs, 1921.

Reinke (1881) that, when the protoplasm of *Aethalium septicum* was treated with a gastric preparation, a large part of it remained undigested. Zacharias (1881, 1883) and Schwarz (1887) observed microscopically that the nucleus and chloroplasts and part of the cytoplasm of plant cells similarly resisted the action of gastric juices. Stutzer (1881) standardized a method of estimating the indigestible proteins by means of a preparation of pig's stomach-lining which Palladin adopted. He found that during the germination of wheat the total proteins in 100 plants diminished; but non-digestible proteins increased. The rate of carbon dioxide release rose also. On four separate occasions the ratio CO_2/non-digestible-N was remarkably constant, the values being 1·05, 1·15, 1·07, 1·18. Other experiments yielded similar ratios with *Lupinus luteus* (1·12) and *Vicia faba* (1·10). The lupin experiment was prolonged into a phase of starvation, in which respirable reserves were depleted, and respiration began to fall off, though the amount of non-digestible protein remained unaltered. The dependence of respiration rate on substrate concentration under mild starvation conditions has also been shown in many subsequent investigations. Palladin's experiment is of great interest in showing that respiration rate is affected by both plastic and non-plastic components of the protoplasm.

Extraction of proteins from leaves has been more recently achieved, and it has, further, proved possible to separate them into chloroplastic and cytoplasmic proteins.[1] It has sometimes been found that leaf respiration per unit dry weight was closely related to the total protein-N on the same basis; but more generally the curve relating the two variables is concave to the respiration axis and does not appear to extrapolate to the origin.[2] Estimating the cytoplasmic protein-N of Sudan grass leaves, Wood (1941) found a closer connexion of both aerobic and anaerobic respirations with cytoplasmic protein than with total protein, and concluded that the former might be regarded as 'a measure of the number of respiratory centres', or, as we have previously named the aggregate, the catalytic protoplasm. Nevertheless, we must remember that there is not one kind of respiratory centre, and that catalytic protoplasm contains many qualitatively different respiratory enzymes, possibly distributed in different organelles. Although these attempts perhaps afford the closest that has yet been attained to a logical basis, their practical difficulties are considerable, even without further refinement.

There is thus no natural unit of respiration, in the sense of a quantity with which all other amounts of respiration may be numerically compared. The amount of respiration performed by a single cell varies with time, place, and tissue; and all other criteria are equally variable. Numerical comparisons can only be achieved within limited series and with a unit chosen for a defined purpose; if it is reasonably satisfactory for this purpose one has to be content.

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[1] See Chibnall, 1939; Wildman and Jagendorf, 1952.

[2] Gregory and Sen, 1937; Richards, 1938; Wood, 1941, 1942.

THE RANGE OF RESPIRATION RATES

It will be realized that it is not easy to compare respiration rates of one tissue with those of another; and that the comparison can only be made on an arbitrary basis. The most commonly used is the Q_{CO_2} or the corresponding Q_{O_2}. Table 1 illustrates the wide variation, of the order 10^6, found in different tissues.

TABLE 1

Respiration in cu. mm. CO_2 *(or* O_2*) per hr. per mg. dry weight*

	Q_{CO_2}	
Yeast	60–100	Meyerhof, 1925
Aspergillus niger		
2-day culture	78	Kostychev, 1927
4-day culture	11·5	" "
Arum italicum		
spadix disks	31	James and Beevers, 1950
peduncle disks	2·9	" " "
Sauromatum		
spadix	30–68	van Herk, 1937
unopened flower	1·6	" "
Tomato		
root tips	6–8	Henderson and Stauffer, 1944
Ulva		
fronds	2·6	Watambe, 1932
Beetroot		
disks	0·38–1·65	Bennet-Clark and Bexon, 1943
Barley		
7-day seedlings	1·6	James and James, 1940
Sunflower		
germinating seeds	1·5	Kidd, West, and Briggs, 1921
3-month plants	0·2	" " "
Peas		
air-dry seed	0·00012	Smith and Gane, 1934
	Q_{O_2}	
Kidney slices	−21·0	Krebs, 1934
Brain slices	−14·0	" "
Liver slices	−9·0	" "
Lung slices	−6·0	" "
Large intestine	−3·0	" "

In connexion with the very high values for yeast and *Aspergillus*, it is well to remember that the fungi respire external substrates whereas those of the higher plants are included within the tissues. Since the dry weight enters into the Q_{CO_2} ratio as divisor, the suggestion of a higher metabolic rate in the fungi is largely illusory. This also has a parallel in some angiosperm tissues, and is illustrated by measurements on *Arum* spadix.[1] The oxygen uptake of a unit volume of tissue glutted with starch was 344 μl./hr. At a later stage when the starch was largely depleted, the oxygen consumption of the same volume fell to 203 μl./hr. The corresponding Q_{O_2} values rose on the other hand from 9·6 to 31·8. It will be noted that, for most higher plant tissues, Q_{CO_2} lies below 2.

Since the respiration of all unitary amounts of living matter varies, the fluctuations may themselves be of prime importance. For this reason, spontaneous respiratory drifts with time, as well as variations with known conditions, have played a very large part in respiration studies. They form the subject of part of this and of the whole of the following chapter.

TEMPERATURE

It was shown at a very early stage of respiration studies that a rise of temperature within the biological range is likely to cause an acceleration of respiration rate.[2] At 0° C. carbon dioxide emission is usually measurable but slow, and an increasing rate may be experienced up to 40° C.[3] or even 50° C.[4] It was sometimes, though not always, found that in the higher ranges the rate declined, and various estimates were made of the point at which the inflexion from acceleration to deceleration took place. This so-called optimum temperature was usually estimated to lie between 35° C. and 40° C.[5] It was, however, realized that, unlike the rates of the lower half of the temperature range, those of the upper were not maintained over periods such as 5–6 hours, and that the higher the temperature became the faster was the decline. The dual nature of metabolic rate-temperature curves was first clearly expressed by Blackman (1905) who separated the primary acceleration, independent of time, from the secondary depression, progressive with time. There are thus two effects to be studied and, as yet, attention has been focused almost wholly on the acceleration.

The temperature at which the secondary depression first perceptibly influences the results generally lies somewhere in the middle of the 0–50° C. range. In the two most thorough-going investigations yet carried out, Fernandes, working with peas, found respiration rates constant over 6 hours up to 30° C.; but Kuijper (1910), with wheat and peas, found depressions at any temperature above 20° C. and with lupins above 25° C. Gerhart (1930) gives the figure 25–30° C. for strawberries. To investigate the primary effect, it is therefore usually necessary to work with temperatures below 30° C. Since a time curve of the secondary depression can be constructed for any temperature by taking readings at successive intervals, it might be possible to obtain corrected readings for 'initial' respiration rates at high temperatures by extrapolating the curves back to zero time. This was successfully done by Blackman (1905) for a series of assimilation results; but Kuijper (1910) found serious difficulties in attempting to apply the method to respiration studies. Since the observed results were summation readings over relatively long time intervals, they could not be satisfactorily extrapolated to give an instantaneous value; nor was the form of the curves such as to make extrapolation mathematically reliable. The same difficulties are also evident in more recent data and, so far as respiration is concerned, it does not seem possible at present to obtain information of value by this method.

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[1] James and Beevers, 1950.

[2] Dehérain and Moissan, 1874; Wolkoff and Mayer, 1874.

[3] Rischavi, 1877.

[4] Kreusler, 1887; Bonnier and Mangin, 1884.

[5] Mayer, 1876; Borodin, 1876; Clausen, 1890; Ziegenbein, 1893.

The temperature coefficient

Within the range 0–30° C. the acceleration of respiration with temperature is usually roughly exponential.[1] If the rise were accurately exponential, the ratio of the rates at any fixed temperature interval would remain constant at whatever position upon the range the interval was taken. A convenient interval in biological work is 10° C., and the ratio, rate at $t+10$° C./rate at t° C., is referred to as Q_{10}. It has been much used as a method of expressing the slope of the rate-temperature curve. As it is readily calculated, easily understood, and carries no theoretical implications, it remains a useful and favourite method of recording the temperature relations of complex biological processes.

It is sometimes objected to the use of Q_{10} that it does not remain constant with rise of temperature even for simple chemical reactions. For an irreversible reaction obeying the Arrhenius equation (p. 28), the Q_{10} drops, over the range 0–30° C., by about 5 per cent., a quantity unlikely to exceed the error of its determination in a biological system. The objection therefore seems of little moment. Much larger variations of Q_{10} exist between different types of process. The principal distinctions can be seen in Table 2. Diffusion rates, whether in water or dilute gels, have Q_{10}'s lying within the very narrow range 1·2–1·3. Heterogeneous chemical reactions (e.g. Cd+I in KI, Table 2) may also have

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[1] Matthaei, 1904; Blackman, 1908; Kuijper, 1910; Gane, 1936; and see data of Rischavi, Clausen, Belehradek and Belehradkova, and Gerhart, 1930.

Q_{10}'s of the same magnitude, the rate of the reaction being set by the relatively slow diffusion to the reactive surface.[1]

TABLE 2

Temperature coefficients within the limits 0–30° C.

	Temperature °C.	Q_{10}	*Reference*
Q_{10} fractional			
Solubility, CO_2 in water	5–15	0·72	Bohr, 1899
Viscosity, 20 per cent. sucrose in water	5–15	0·72	
$Q_{10} = 1·0–1·2$			
Photochemical:			
oxidation of alcohol by $(NH_4)_2Cr_2O_7$	20–30	1·0	Plotnikov, 1919[2]
decomposition of NaOCl	10–24	1·06	Spencer, 1914[2]
$2\,HI + O_2 \rightarrow I_2 + 2\,H_2O$	15–25	1·18	Berthoud and Nicolet, 1927
$H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2$	10–30	1·15	Tian, 1916[2]
bromination of stilbene	0–20	2·1	Berthoud and Beroneck, 1927[2]
decomposition of $K_3Co(C_2O_4)_3$	12–22	1·06	Griffith and McKeown, 1929
$Q_{10} = 1·2–1·3$			
Diffusion:			
KCl in agar gel	20–30	1·22	Mann, 1924
mean for aqueous solutions	18	1·24	Moelwyn-Hughes, 1933
Conductivity, KCl solution	15–25	1·23	Kohlrausch[3]
Heterogeneous chemical reaction, Cd+I in KI	15–25	1·30	Van Name, 1917[4]
$Q_{10} = 1·4–2·0$			
Enzyme catalysis:			
$H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2$ (catalase)	0–20	1·4	Nordefeldt, 1920
maltose hydrolysis (maltase)	10–20	1·90	Lintner and Kröber, 1895
starch hydrolysis (amylase)	20–30	1·96	Luers and Wasmund, 1921
$Q_{10} > 2·0$			
Chemical reactions:			
$H_2O_2 + I' \rightarrow H_2O + IO'$	15–25	2·2	Walton[4]
mutarotation of glucose	5–15	3·08	Hudson and Dale[4]
decomposition of $K_3Co(C_2O_4)_3$	12–22	4·56	Griffith and McKeown, 1929
$N_2O_5 \rightarrow N_2O_4 + \frac{1}{2}O_2$ in gas	25–35	4·00	Moelwyn-Hughes, 1933
$N_2O_5 \rightarrow N_2O_4 + \frac{1}{2}O_2$ in CCl_4	25–35	3·96	” ”
$N_2O_5 \rightarrow N_2O_4 + \frac{1}{2}O_2$ in $CHCl_3$	25–35	3·90	” ”
Enzyme catalysis:			
peptone hydrolysis (erepsin)	15–25	2·6	Vernon, 1903
$Q_{10} \gg 2·0$			
Enzyme inactivation:			
peroxidase	70	3,040	Zilva, 1914[5]

Chemical reactions of normal thermal types show much higher and more diverse Q_{10}'s. They rarely fall below 2 and reach 4 or higher. It is noteworthy that reactions in various solvents, or even in a gas phase, are not distinguished by different ranges of Q_{10}, the relatively small effect of temperature on viscosity not causing more than a small fractional rise. Enzyme-catalysed reactions have Q_{10}'s somewhat lower than the corresponding reaction uncatalysed or catalysed by H^+ ions.

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[1] Nernst, 1923.

[2] Quoted from Griffith and McKeown, 1929.

[3] Quoted from Eggert, 1932.

[4] Quoted from Moelwyn-Hughes, 1933.

[5] Reactions with very high Q_{10}'s are too slow to be detectable in the range 0–30° C.

Photochemical reactions are often independent of temperature and have Q_{10}'s approximating to 1·0; but occasionally rising to 1·5 or higher (Griffith and McKeown, 1929). Where the Q_{10} exceeds unity and it is clear that the characteristics of a photochemical reaction are being determined, and not those of an accompanying thermal reaction, it can be concluded that preliminary thermal activation is needed before addition of light quanta can cause reaction to occur. An example in which a relatively large thermal increment is needed is shown in the bromination of stilbene (Table 2). Where a reaction, such as the decomposition of potassium cobaltioxalate, $K_3Co(C_2O_4)_3$, can occur either thermally or photochemically, the Q_{10} of the photoreaction is always lower than that of the purely thermal reaction, even if the former is greater than unity.

It is sometimes overlooked that, in determining the overall rate of a series of reactions including one or more that are reversible, the rates of the back reactions may be as important as those of the forward reactions. In the series $A \rightleftharpoons B \rightarrow C$ the rate of formation of C depends upon the relative rates, and hence the equilibrium in $A \rightleftharpoons B$. This will be displaced with change of temperature according to Le Chatelier's principle that, a constraint having been placed upon the equilibrium, it will undergo readjustment tending to nullify the constraint. If the reaction is endothermic in the direction of $A \leftarrow B$, a rise of temperature will tend to reduce the concentration of B and hence of the production of C. The Q_{10} of the overall reaction $A \rightarrow C$ will then be depressed, and may even become fractional. Respiratory examples under this heading are given on pp. 33 et seq. Solubility equilibria (Table 2) may also affect the observed Q_{10} of respiratory gaseous exchange.

Temperature coefficients of respiration within the approximate interval 5–15° C. are listed in Table 3 for a variety of plant tissues. They fall between 2 and 3, showing a very large effect of temperature upon respiration rates, comparable with the effect upon thermal chemical reactions. The conclusion may be drawn that the rate of respiration is normally determined at these temperatures by a chemical reaction and not, for example, by the rate of diffusion of oxygen into the respiring tissues or of a respiratory substrate to its enzyme. This conclusion is not at variance with the Nernst rule that the rate of heterogeneous reaction may be limited by diffusion, but indicates that respiration depends upon 'time' reactions that are chemically slow. It is in agreement with experiments on the internal atmosphere of tissues and those showing that respiration rate is independent of surface area (p. 74). It also agrees with the fact that respiration rate can often be shown to depend on the concentration of some internal substrate such as sucrose[1] or amino acids.[2]

TABLE 3

Temperature coefficients within the range 5–15° C. (approx.)[3]

	Range	Q_{10}	
SOAKED GRAINS			
wheat	5·0–15·0	2·2	Janerka, 1912
SEEDLINGS			
Lupinus luteus	5·0–15·0	2·6	Kuijper, 1910
" "	5·0–15·0	2·5	Clausen, 1890
wheat	5·0–15·0	2·4	" "
" 3-day	5·0–15·0	2·4	Kuijper, 1910
" etiolated	5·0–15·0	3·0	Rischavi, 1876
peas	5·0–15·0	2·8	Kuijper, 1910
COLEOPTILES			
oat, 72 hours	10·0–15·0	1·9	Bottelier, 1930
" 96 hours	10·0–15·0	2·6	" "
BUDS			
lilac	5·0–15·0	2·4	Clausen, 1890
asparagus	10·0–14·0	2·5	Platenius, 1942
LEAVES			
blackberry	7·5–15·8	3·1	Kreusler, 1887
spinach	10·0–14·0	2·6	Platenius, 1942
lettuce	10·0–14·0	2·0	" "
FRONDS			
Scolopendrium (young)	5·0–15·0	3·5	Belehradek and Belehradkova, 1929
" (old)	5·0–15·0	1·6	Belehradek and Belehradkova, 1929
ROOTS			
carrots	10·0–14·0	1·9	Platenius, 1942
"	7·0–13·0	3·2	Robertson, 1944
FRUITS			
strawberries	5·0–15·0	2·5	Gerhart, 1930
bananas	5·0–15·0	2·4	Gane, 1936
pawpaws	4·5–15·5	3·0	Jones, 1942
tomatoes	10·0–14·0	2·3	Platenius, 1942
cucumbers	10·0–14·0	1·9	" "

When a series of Q_{10} values from low to high temperatures is obtained, it frequently shows that the Q_{10} is not constant, but falls away much more than 5 per cent., usually more or less smoothly. An exception was noted by Wager (1941) in *Cassiope hypnoides* with a constant $Q_{10} = 2{\cdot}1$ between 0–40° C.

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[1] Barker, 1936.

[2] Wood, 1941, 1942; Gregory and Sen, 1937; Richards, 1936.

[3] Q_{10} calculated where necessary on the assumption $Q_{10} = (R_{t_2}/R_{t_1})^{10/(t_2-t_1)}$.

TABLE 4

Temperature coefficients from 0 to 55° C.

Temperature interval	*0–10*	*5–15*	*10–20*	*15–25*	*20–30*	*25–35*	*30–40*	*35–45*	*40–50*	*45–55*	
Wheat .	..	3·0	2·4	1·8	1·7	1·6	1·7	..	..	..	Rischavi, 1876
	2·9	2·4	2·1	1·9	1·6	1·2	1·1	0·9	0·6	0·1	Clausen, 1890
	2·6	2·4	2·3	1·9	1·8	1·3	1·1	1·0	0·8	..	Kuijper, 1910
	..	2·2	..	1·8	..	..	..	..	..	..	Jauerka, 1912
Peas .	3·0	2·8	2·4	2·3	1·8	1·6	1·4	1·1	1·0	0·5	Kuijper, 1910
	3·3	..	2·5	..	1·7	1·3	1·2	1·2	0·6	..	Fernandes, 1923

Temperature interval	*0–10*	*10–20*	*20–30*	*30–40*	
Empetrum nigrum					
Shoots (Arctic)	3·6	2·7	2·2	..	Wager, 1941
,, (Dublin)	3·5	2·7	2·3	2·0	,, ,,
Saxifraga oppositifolia					
Shoots (Arctic)	3·0	2·7	2·2	1·9	,, ,,
,, (Dublin)	3·9	2·5	2·1	1·8	,, ,,

Kuijper's results for wheat, peas, and lupins are also illustrated in Fig. 1. There is no obvious inflexion in any of these series at 20–30° C., the temperature at which a falling away with time becomes apparent in the rates. Roughly speaking, the Q_{10} is always in the neighbourhood of 3 around 10° C. and in the neighbourhood of 2 at 20–30° C., a temperature range in which secondary depression has little effect. The rates of all the partial reactions are likely to be affected by temperature in varying degrees, and the observed Q_{10} of CO_2 emission (or O_2 uptake) will be most affected by the slowest reactions, i.e. at low temperatures by the reactions having the highest Q_{10}'s and at high temperatures by the reactions having the lowest Q_{10}'s.[1] It is therefore not at all surprising to find that a falling series of Q_{10} values is obtained, even apart from secondary effects at extremes. Furthermore, the Q_{10}'s of hydrolyses catalysed by enzymes *in vitro* often show a similar falling away, and it is possible that, at temperatures around 30° C., substrate diffusion is beginning to exercise a restraint upon the rate of these heterogeneous reactions whether isolated *in vitro* or during respiration *in situ* in the cell. By contrast, the oxidizing enzymes and invertase, which behaves in other ways as though giving virtually a true solution, do not show such falling Q_{10} values until inactivating temperatures are reached.

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[1] Cf. the analysis of the temperature coefficient of photosynthesis by Wohl, 1940.

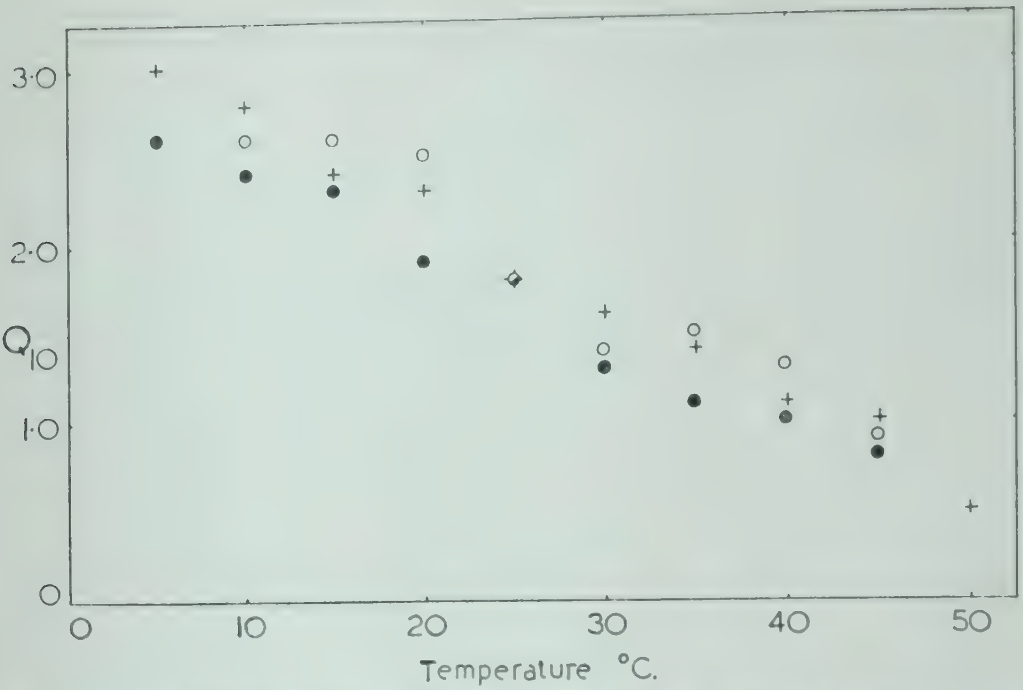

FIG. 1. Q_{10}'s of wheat ●, peas +, and lupins ○, from the data of Kuijper, 1910, for the first hour of respiration.

Energy of activation

An alternative method of expressing temperature relations is by means of the characteristic, E, in the Arrhenius equation:

$$\log_e \frac{k_2}{k_1} = \frac{E}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right).$$

In a simple chemical reaction, E remains constant and has the meaning of energy of activation, i.e. energy increment, in excess of the average molecular energy, necessary for reaction. It varies in different reactions from about 4,000 to 40,000 cal. per gm. molecule. Since the velocity constants k_1 and k_2 appear only as a ratio, they may be expressed in any units, and E can be obtained from the ratio observed at any two temperatures, or, preferably, from a series of temperatures. It has been suggested that the apparent energies of activation calculable by these means from biological data may be of value in identifying the nature of the reaction controlling the rate of the process.[1] The constancy of E can conveniently be tested by plotting $\log_e R$ against $1/T$ (absolute) throughout a range of temperatures[2] to obtain a straight line. The results of Kuijper (1910) for pea and lupin seedlings, at temperatures which Kuijper considered to cause no secondary fall of the rate, are given in this form in Fig. 2, and can be fitted with straight lines, $E = 14{,}700$ (lupins) and $E = 15{,}800$ (peas). Crozier regarded the data for peas as falling on two intersecting straight lines with $E = 16{,}500$ below 10° C. and $E = 13{,}800$ above 10° C.; but this interpretation depends on a single low value at 5° C., and cannot be applied to other results such as those of lupins in the same figure.

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[1] Crozier, 1924; Wohl, 1940.

[2] The temperature interval available biologically (0–30° C.) is too small for very stringent testing.

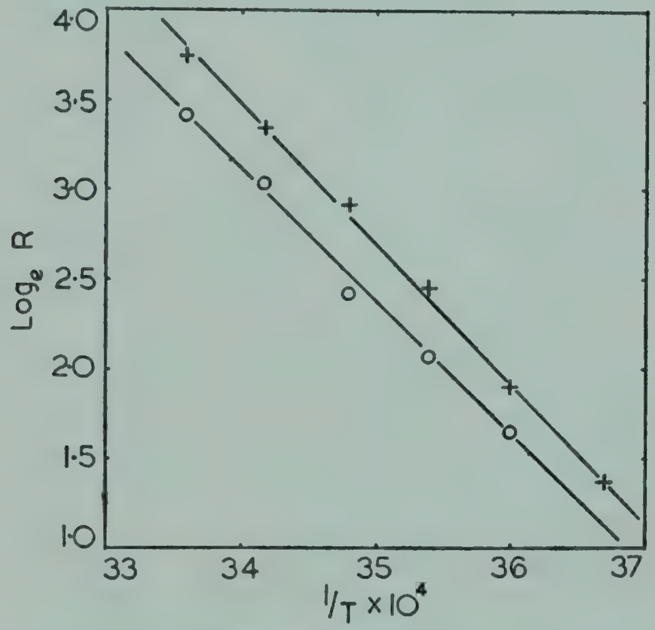

FIG. 2. $\mathrm{Log}_e R$ plotted against $1/T$. Peas +, lupins ○. The slope drawn for peas corresponds with $E = 15{,}800$, for lupins $E = 14{,}700$ cal. per gm. mol. Based on the data of Kuijper, 1910.

It seems unlikely that there should in fact be a sharp change of controlling reactions at about 10° C., and a gradual curve is more probable. If values of E are calculated for an overlapping series of 10° intervals, as for the Q_{10}'s in Table 5, the effect of this single aberrant value is minimized, and a continuous series of diminishing values is obtained.

TABLE 5

Respiration of peas 0–30° C.

Temperature interval	*Kuijper*		*Fernandes*	
	Q_{10}	E	Q_{10}	E
0–10	3·0	16,800	3·3	18,500
5–15	2·8	16,350	..	..
10–20	2·4	14,600	2·5	14,100
15–25	2·3	14,300	..	..
20–30	..	..	1·7	9,400

It is evident that the generalized slopes of Fig. 2 are meaningless as activation energies, and the attempt to analyse them into components within so short a temperature range is too uncertain with the data at present available to be of any value.[1] Even if it were possible to obtain data which might be supposed to belong to individual reactions *in vivo*, there would still be considerable difficulty in identifying them with the corresponding reactions as isolated. Even in simple reactions, E varies with the conditions of reaction.[2] In extracted enzyme systems it is known to vary with acidity and temperature. Fairly consistent values have been obtained by different authors for the relatively simple case of invertase. Summarizing these, Nelson and Bloomfield (1924) concluded that the energy of activation of its hydrolysis of sucrose falls off in accordance with $E = 11{,}400\text{–}100t$, t being the centigrade temperature. The fall seems unlikely in this reaction to be due to diffusion and, according to Sizer (1938), can be avoided if measurements of reaction rate are fast enough to eliminate mutarotation of the glucose formed. If simplified systems give values which are liable to such changes with alteration of conditions *in vitro*, it is evident that they cannot be expected to give recognizable values when incorporated *in vivo*.

The majority of biological reactions cannot be shown to conform to the simple conditions under which E has a definite physical significance,[3] and as an empirical method of expressing the coarse distinctions at present possible with biological temperature data it has no obvious advantages over Q_{10}. On the other hand, it has the obvious disadvantage of being liable to misunderstanding. If the method were applied to the data of Müller-Thurgau (1882) for the respiration of sweetened potatoes, the apparent critical increment, calculated from the transition from 10 to 20° C. would be zero (an unknown type of reaction); and from the data for the reverse shift, 20 to 10° C., it would be 11,500. It is highly probable that at both temperatures breakdown of sucrose (cf. p. 33) is the rate-controlling reaction, and that the anomaly arises from a temperature shift of the starch $\rightleftharpoons$ sugar equilibrium. In this particular example the inapplicability of the method is obvious.

Variation of Q_{10} with age

No significant variations of Q_{10} between different types of material are evident in Table 3, even when determined by a single author using a standardized method. There is, however, some evidence that the Q_{10} of a given tissue varies with its age. Bottelier (1939) found a perceptible rise in oat coleoptiles between the ages of 72 and 96 hours at all temperatures between 10–15° C. and 25–30° C., and especially at the lower end of the range. Differences at the higher end of the range are probably not significant. Changes in ripening fruit appear to be very small, with perhaps a slight drop at the lower temperature intervals.

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[1] Cf. Stearn, 1949.

[2] See e.g. Moelwyn-Hughes for effect of solvent.

[3] Moelwyn-Hughes, p. 50, &c.

TABLE 6

Q_{10} and age

Oat coleoptiles[1]			*Apples*[2]		
Temperature interval	*72 hr.*	*96 hr.*	*Temperature interval*	*At picking*	*Climacteric*
10–15° C.	1·9	2·6	2·5–10·0° C.	2·7	2·2
15–20	1·8	2·2	10·0–22·5	2·2	2·4
20–25	1·8	2·1			
25–30	1·7	1·8			
Bananas[3]			*Scolopendrium fronds*[4]		
Temperature interval	*Unripe*	*Climacteric*	*Temperature interval*	*Very young*	*Old*
0·0–12·5° C.	2·2	1·8	5–15° C.	3·5	1·7
5·0–15·0	2·4	2·4	15–25	1·9	1·7
12·5–20·0	2·4	..			

A series of results for the fronds of *Scolopendrium* obtained by Belehradek and Belehradkova (1929) are more striking. As the temperature intervals used were irregular, their data are most conveniently summarized as log curves (Fig. 3) from which the Q_{10}'s given in Table 6 may be obtained by interpolation. Again, at the higher temperatures there appears to be little difference; but a more pronounced temperature effect becomes apparent with young fronds at the lower temperature intervals. The change of Q_{10} with age is unlikely to be caused by changes of resistance to diffusion in the tissues, since the values remain too high. Direct confirmation of this was obtained by Bottelier, so far as oxygen diffusion is concerned, by measuring Q_{10} of oat coleoptiles in air and in pure oxygen. There was no change.

Q_{10} of anaerobic respiration

Attempts have also been made to determine the effect of withdrawal of oxygen (i.e. elimination of aerobic oxidation stages) upon Q_{10}. Chudiakov (1894) carried out an extensive series of determinations upon germinating seeds and young seedlings of wheat, lupins, and peas, under hydrogen. He was able to show that anaerobic respiration also showed an approximately exponential increase with rising temperature over the range 0–30° C. Kuijper (1910) later determined the Q_{10} of aerobic

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[1] Data of Bottelier, 1939.

[2] Kidd and West, 1930.

[3] Gane, 1936.

[4] Belehradek and Belehradkova, 1929.

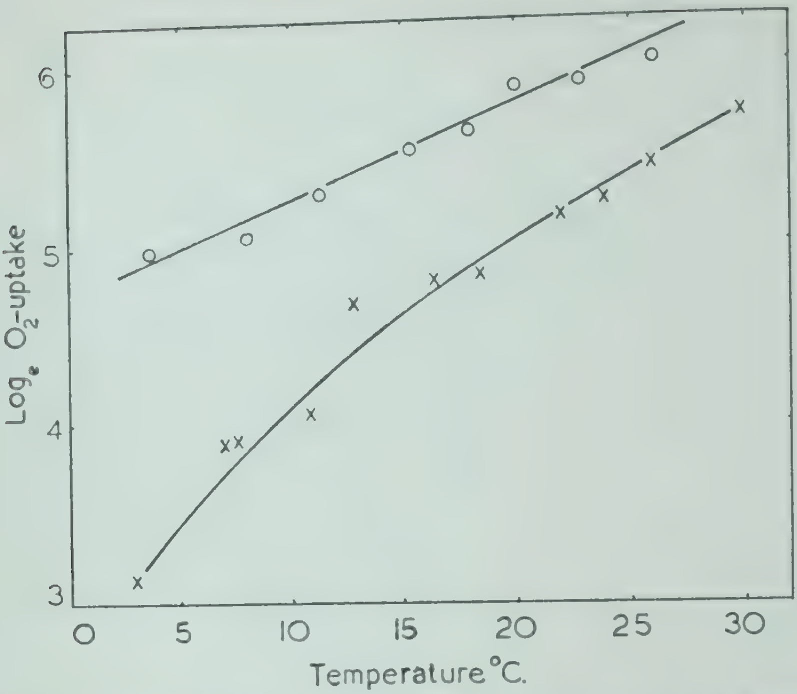

FIG. 3. Log_e oxygen consumption of *Scolopendrium* fronds at 3–30° C. Very young fronds × ; old fronds ○. Based on the data of Belehradek and Belehradkova, 1929.

respiration for seedlings of the same three species. The two sets of results are compared in Table 7 and show remarkable similarities.

TABLE 7

Temperature interval	*Wheat*		*Lupinus luteus*		*Peas*	
	Aerobic	*Anaerobic*	*Aerobic*	*Anaerobic*	*Aerobic*	*Anaerobic*
10–20° C.	2·3	2·3 2·2	2·6	2·8 2·6	2·4	2·6 2·6
20–30° C.	1·8	1·8 2·0	1·8	1·7 1·8	1·8	1·7 1·8

Aerobic data from Kuijper, 1910; anaerobic data from Chudiakov, 1894.

Fernandes (1923) carried out a direct comparison with a batch of germinating peas divided into two equal halves, and only small and irregular differences were found. Chudiakow also observed CO_2-emission rates during transfer from air to hydrogen and back to air. Over the short periods used in his experiments there was no pronounced drift of the CO_2-emission rates, and he was able to calculate satisfactorily I/N (anaerobic/aerobic) ratios; and found that they remained independent of temperature.

These close numerical relations between aerobic and anaerobic respiration suggest that at moderate temperatures the rate of the process is usually restricted by the common anaerobic phase. Bottelier found that the Q_{10} of oat coleoptiles was reduced by dilute cyanide, which appears at first sight to be at variance with this conclusion; but oxidation of triosephosphate, which occurs during anaerobiosis, appears to be restricted by cyanide,[1] and may account for the cyanide effect at moderate concentrations.

Thermal displacement of equilibria

A reversible chemical reaction consists of two opposed reactions which are thermal opposites. In the equation

$$\underset{\text{in starch}}{(C_6H_{10}O_5)} + H_2O \rightleftharpoons C_6H_{12}O_6$$

reaction towards the right liberates 3700 cal of heat for each gm. molecule of glucose anhydride released as glucose. Reaction towards the left would absorb the same amount of heat into the system, and the equilibrium reached depends upon the temperature. Qualitatively (which is all that has much present significance in biology) the equilibrium constant rises with temperature, i.e. the transformation is displaced in favour of the left-hand side, high temperatures favouring the formation of starch, low temperatures that of sugar.

Starch—sugar

The transformation of starch to sugar is likely to be more than a single reversible reaction; but the heat relations of the whole cycle must be such that passage from starch to sugar-complex is exothermic. It was early realized that the sweetening of potatoes exposed to low temperatures was due to a temperature-induced shift of the starch-sugar equilibrium, and that considerable modification of the respiration rates resulted. Müller-Thurgau (1882) made an extensive investigation, the results of which have been confirmed and extended by subsequent workers as more discriminating methods of carbohydrate analysis have become available.[2] He found that when potatoes were transferred from

TABLE 8

Reducing sugar in potatoes

Data of Müller-Thurgau, 1882

Temperature, °C.	0	3	6	8	10
Reducing sugar, per cent. fresh weight	2·35	0·65	0·35	traces	nil

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[1] James, Heard, and James, 1944.

[2] See for example, Müller-Thurgau and Schneider-Orelli, 1910; Butler, 1913; Hopkins, 1924; Appleman, 1911; Barker, 1936; Wolff, 1926.

room temperature to temperatures below 8° C., reducing and non-reducing sugars both accumulated, at first slowly and then through a sigmoid time curve of increment to an equilibrium. He was able to show that loss of starch occurred at the same time. The reverse transition, from low to normal temperatures, brought about the opposite

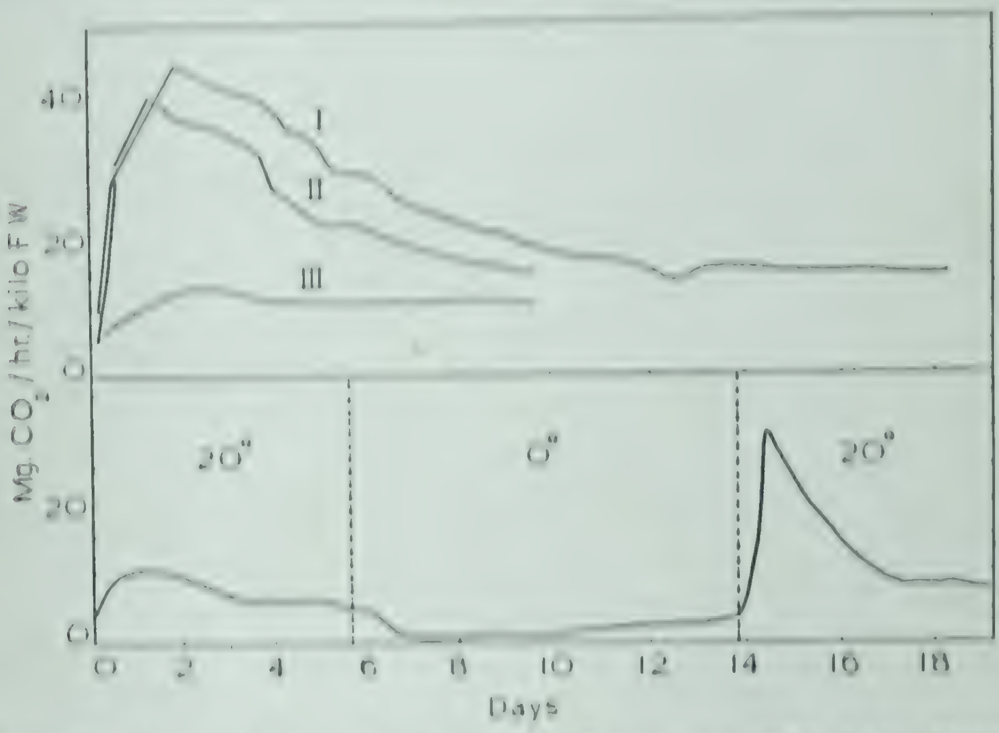

FIG. 4. Respiration of potatoes at 0° and 20° C. Top curves at 20° C. I after 26 days at 0° C., II after 13 days at 0° C., and III without previous cooling. The lower curve shows the course of CO_2 emission during transfers from 20° C. to 0° C. and vice versa. Data of Muller Thurgau, 1882.

shift of the carbohydrate equilibrium. Sugar rapidly disappeared in excess of that equivalent to the CO_2 of respiration, and as there was no significant amount of growth, the difference could only have been converted back to starch.

Respiration rates at temperatures above 8° C. were found to be normally restricted by the low concentration present. After a period at 0° C., the respiration rate at 20° C. was raised 300–400 per cent. It then fell slowly back to the normal rate for the temperature as the sugar formed at the low temperature disappeared from the tissues (Fig. 4). In experiments where potatoes were carried through a series of temperature changes, the form of the respiration drift at any given temperature was determined by the previous history. Upward changes of temperature were always accompanied by a temporary peak of CO_2 output, whereas downward changes showed only a drop to the new level without a temporary acceleration (Fig. 4). These differences are consistent with the starch—sugar shifts described. In the experiments of Hopkins (1924), the shift between 0° and 4·4° C. was so pronounced that the rate of CO_2-emission was actually faster at the lower temperature.

TABLE 9

Potatoes at 20° C. after cooling

Data of Müller-Thurgau

Cooling	*Loss of sugar (gm.)*	*Total* CO_2 *emission (gm. hexose equivalent)*	*Drift of* CO_2 *emission*
26 days at 0° C.	90·950	40·416	Fig. 4, Curve I
13 days at 0° C.	69·953	22·240	Fig. 4, Curve II
nil	. .	14·100	Fig. 4, Curve III

In an elaborate reinvestigation, Barker (1933) confirmed these relations and later showed that the effective sugar was sucrose (1936). Since there was a close connexion between sugar concentration and the rate of CO_2-production (see p. 108), the reactions investigated might be summarized: starch $\rightleftharpoons$ sugar $\rightarrow CO_2$; but Barker's identification of sucrose as the sugar concerned shows that the starch $\rightleftharpoons$ sugar transformation cannot be a simple hydrolysis.

A previously unreported effect was also revealed in Barker's results in the form of a persistent depression of respiration caused by exposure to low temperatures. It lasted for several weeks after return to a higher temperature, and was attributed to the accumulation of an inhibitor. Although the appearance of the inhibitor was not initiated above about 10° C., the rate of its formation could be greatly accelerated by temporary exposure to 15° C., when respiration rates might be depressed as much as 26 per cent. in 24 hours. As the amount of depression depended on previous as well as current temperatures, very different amounts were found in individual records, varying from a complete absence of depression to about 50 per cent.

A temperature shift of the starch $\rightleftharpoons$ sugar equilibrium also occurs in maples and other trees, resulting in a rapid acceleration of bleeding when the temperature drops (Meeuse, 1949).

Fat—carbohydrate

The respiration of acorns has been investigated at different temperatures.[1] The rise of carbon dioxide production was described as being usually linear with temperature rather than exponential; i.e. there was a marked fall of Q_{10}. In one experiment with White Oak, it fell from 2·6 at 5–15° C. to 1·8 at 20–30° C. Oxygen consumption showed altogether different relationships. It was relatively fast at low temperatures, and showed little increase over the range 5–30° C.; and there was often an actual reduction of oxygen uptake in the middle of the range. As a result, the R.Q. started at very low values, 0·1–0·2 at 0° C. and rose to 0·5–0·7 at 30° C. The very low R.Q. at low temperatures can only be explained by an incomplete oxidation which is, no doubt, the conversion of fat → sugar familiar in the germination of fatty seeds (see p. 93). Fats are likely to have higher heats of formation than carbohydrates. For example, in the conversion of triolein (olive oil) to hexose,

---

[1] J. W. Brown, 1939.

$$2\ C_{57}H_{104}O_6 + 10\ H_2O + 46\ O_2 \rightarrow 19\ C_6H_{12}O_6$$

assuming a heat of combustion of olive oil of 9·5 kcal per gm., the heat of reaction $-\Delta H = 5613$ kcal per mol. triolein; i.e. is strongly exothermic. A rise of temperature would, therefore, be expected to swing the fat—carbohydrate equilibrium towards the left, with a corresponding reduction of oxygen consumption as actually observed. It would also account for the relatively small increase of CO_2-production (falling Q_{10}) by limiting the sugar concentration.

Temperature relations of malate formation

Bennet-Clark (1933) found that the amount of malate accumulating in detached and darkened leaves of *Sedum praealtum* was a function of temperature; more acid accumulated at 3° C. than at 27° C. Later, Thomas and Ranson[1] also found that the titratable acids in similarly treated *Bryophyllum* leaves were more abundant at 12° C. than at 32° C. (Fig. 5). Bennet-Clark explained his results in similar terms to the previous paragraphs, on the assumption of a carbohydrate—malate equilibrium, exothermic in the direction of malate formation. It is now, however, known that malate formation is coupled with CO_2-fixation as well as carbohydrate loss. The equation proposed[1]

$$C_6H_{12}O_6 + 2\ CO_2 \rightleftharpoons 2\ C_4H_6O_5$$

is endothermic to the extent of 16·7 kcal per mol. hexose in the direction of malate formation. If adjustment of this equilibrium were the governing factor, a drop of temperature would be expected to lower acid concentration.

Thomas and his collaborators have shown that the acid formation is a function of CO_2-concentration over the range 0–10 per cent. In the light, CO_2-concentration is normally kept very low owing to the high affinity of CO_2 for its photosynthetic acceptor, and acid disappears. In the dark it is formed from respiratory CO_2, and its formation enhanced by raising the external CO_2-concentration. It has been further shown that CO_2-fixation as well as acid formation is increased by lowering the temperature, and that the two always coincide in time with perhaps a slight lag of acid formation.

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[1] Thomas, 1947, 1949, 1951; Thomas and Beevers, 1949.

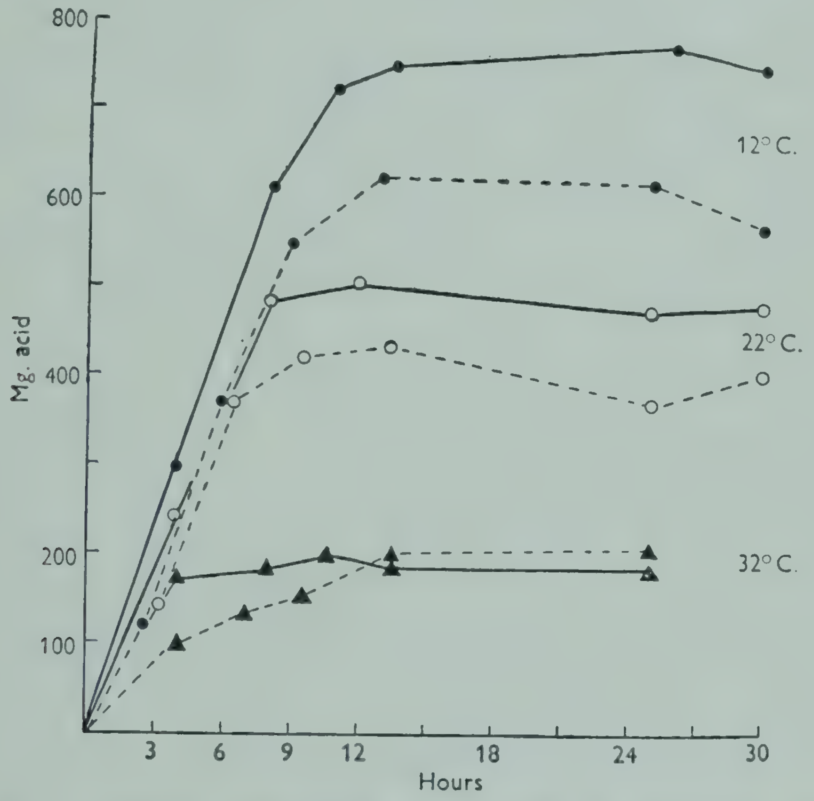

FIG. 5. Increase of titratable acid (broken lines) in *Bryophyllum* leaves kept in the dark in 5 per cent. CO_2 at 12, 22, and 32° C. The acid equivalent of the CO_2 simultaneously fixed (assuming $C_6H_{12}O_6 \rightarrow 2C_4H_6O_5$) is shown by the continuous lines. Ranson's data, after Thomas, 1951.

It is improbable that sugar as such reacts with CO_2, and the most probable sugar derivative to do so is pyruvate. Even so, the combination of pyruvic acid with CO_2 to give malic acid by way of oxaloacetic is still a highly endothermic reaction. It also requires a large increase of free energy and is of improbable occurrence in isolation. Ochoa (1946) suggested that it might occur by coupling with a strongly exergonic reaction, such as the oxidation of Coenzyme I, when catalysed by appropriate enzymes. The observed free energy change in the carboxylation reaction

$$CH_3 \cdot CO \cdot COO^- + HCO_3^- \rightarrow {}^-OOC \cdot CH_2 \cdot CO \cdot COO^- + H_2O$$

is $+\Delta F = 5250$ cal.[1] From the known redox potentials, Ochoa calculated

$$\text{Oxaloacetate} + \text{CoI}\cdot\text{H}_2 \rightarrow \text{malate} + \text{CoI}$$

to have $-\Delta F = 8300$ cal, so that the overall conversion of pyruvate + bicarbonate to malate, with oxidation of Co I.H_2 to Co I would proceed with $-\Delta F = 3050$ cal. Two enzymes are involved, oxaloacetic carboxylase in the first partial reaction and malic dehydrogenase in the second. Vennesland *et al.*[2] have demonstrated the existence of the former in a considerable variety of plant tissues, and found it to be always accompanied by a malic dehydrogenase which they found to co-operate with Coenzyme II (not Coenzyme I; cf. Table 30, p. 187). The method of co-operation of the two enzymes, or indeed whether one enzyme combines the two functions, is still uncertain; but it appears likely that the somewhat unexpected temperature relations of malate formation may result from their being dominated by the overriding Coenzyme II oxidation.

Thermal depression of respiration rates

The main question to be settled is whether specific inactivation of any part of the respiratory system takes place before general breakdown of the protoplasm sets in. If a definite temperature optimum were observed, it could be explained in this way; but the earlier assumptions of its existence have proved fallacious (p. 22). The thermal depression of respiration has a very high Q_{10}. Data for the interval 40–50° C. can be calculated from Kuijper's results.

TABLE 10

Thermal depression of respiration

Species	*Time interval (hr.)*	*Temperature interval*	Q_{10}
Pisum sativum . . .	$\frac{1}{4}$–$\frac{1}{2}$	40–50	2·5
" " . . .	$\frac{1}{2}$–1	40–50	5·5
" " . . .	$\frac{1}{2}$–2	40–50	3·1
" " . . .	1–2	40–50	2·0
" " . . .	1–2	35–45	4·3
Triticum sativum . .	1–2	35–45	2·3
" " . .	1–2	40–50	3·7
Lupinus luteus . . .	1–2	40–50	4·6

The Q_{10} of inactivation of emulsin at 49–59° C. is 8·2,[3] and of saccharase at 54–74° is 98.[3] Zilva (1914) obtained $Q_{10} = 3040$ for a milk peroxidase at 69° C.

---

[1] Evans, Vennesland, and Slotin, 1943.

[2] Vennesland, Gollub, and Speck, 1949; Conn, Vennesland, and Kraemer, 1949.

[3] From data collected in Haldane, p. 69. See also Sizer, 1943.

At 40° C. and upwards Kuijper observed rapid and continuous deceleration of CO_2-output. Although Kuijper's time curves of inactivation have been referred to as logarithmic, they depart markedly from such a course (Fig. 6); there is a progressive slowing of deceleration, and at moderate temperatures total inactivation is indefinitely delayed. If we are to suppose that the reduction of respiration rate depends upon the inactivation of the most thermolabile enzyme, it would appear that the process cannot be kinetically unimolecular. The heat inactivation of some extracted enzymes is known to follow such a simple course;[1] but Euler and Laurin (1919) found that the unimolecular 'inactivation constant' of invertase held at 59° C. fell away somewhat in the fashion described above for respiration. The heat stability of enzymes is also known to be much affected by the presence of accompanying proteins, the acidity at which they are held, presence of substrate, &c. While there is a general similarity between the retardation of respiration and the inactivation of enzymes, it is clearly impossible at present to make any close comparison between the two.

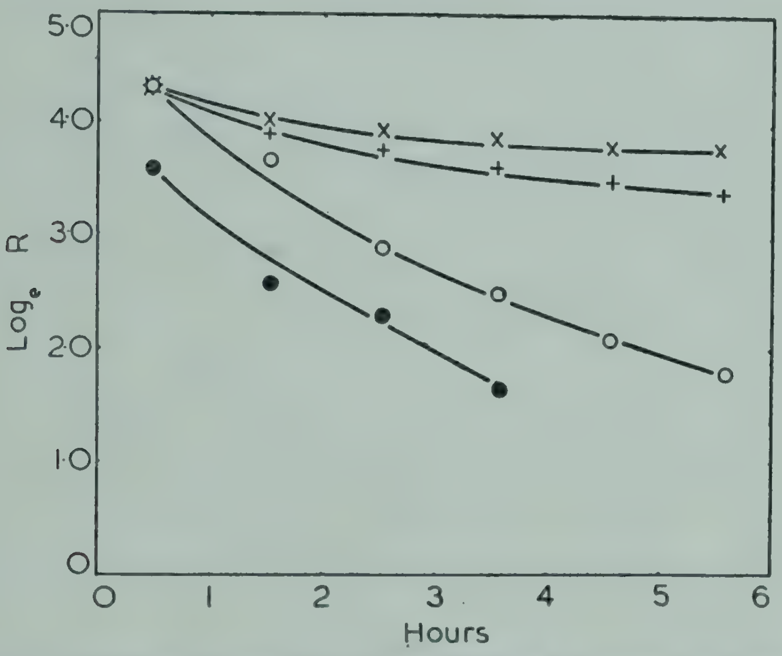

FIG. 6. Logarithms of respiration of peas during 6 hours at inactivating temperatures; ×, 40° C.; +, 45° C.; ○, 50° C.; ●, 55° C. Based on the data of Kuijper, 1910.

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[1] See Euler, 1920; Haldane, 1930.

III

RESPIRATORY DRIFTS

MUCH of the pioneer work on plant respiration depended on measurements of CO_2-output, and a favourite method of working was to sweep the carbon dioxide out of the respiration chamber into a Pettenkofer absorption tube by means of a continuous air-stream. About the turn of the century, F. F. Blackman devised a switch which automatically diverted the gas-stream through a series of Pettenkofer tubes at fixed intervals. He applied it to the investigation of CO_2-outputs of tissues, prolonged over intervals of weeks or months, and so initiated what has proved to be one of the major approaches to the study of plant respiration and the basis of modern fruit storage.

DETACHED LEAVES

The first respiration drifts to be examined in detail were those of detached leaves of cherry-laurel (*Prunus laurocerasus*). The choice was dictated by experimental convenience, and the leaf was chosen as one that would not deteriorate too rapidly when isolated. The records were followed through until obvious necrosis set in, and the characteristic spontaneous drift thus made known became the starting-point for further manipulation. The normal conditions of experiment confined mature cut leaves in a dark chamber with their petioles dipping into water and a continuous current of air passing through at a temperature around 20° C. The complete time curve of CO_2-emission is represented in Fig. 7[1] for leaves that were maintained at 16·5° C. Most subsequent experiments have been run a little warmer. Blackman analysed this curve into six phases—characterized as follows:

Phase	*Duration in days*	*Slope*	*Colour of leaves*
1	1	high level	green
2	6	rapid fall	green
3	6	low level	green → pale green
4	c. 20	steady rise	pale green → yellow
5	c. 20	steady fall	yellow → brown
6	. .	sharp rise	brown

---

[1] This figure has not been previously published. It is a reduction of a wall-diagram designed by the late Dr. F. F. Blackman to illustrate his results and used by him in lectures to the Cambridge Botany School to whom I am indebted for permission to use it here. Much of the work on cherry-laurel has not been published in any journal and the remarks above are based on Dr. Blackman's lectures and on personal communications.

Before attempting any analysis of the metabolic processes underlying these changes, it will be well to compare them with corresponding results for other species. Blackman chose *Tropaeolum majus* leaves as a contrasting type, and their time curve of CO_2-emission is also represented in Fig. 7. The form of the curve is very similar to that for cherry-laurel; but the drift is much faster, and, to make the successive phases correspond, the time axis for *Tropaeolum* has been expanded twofold.

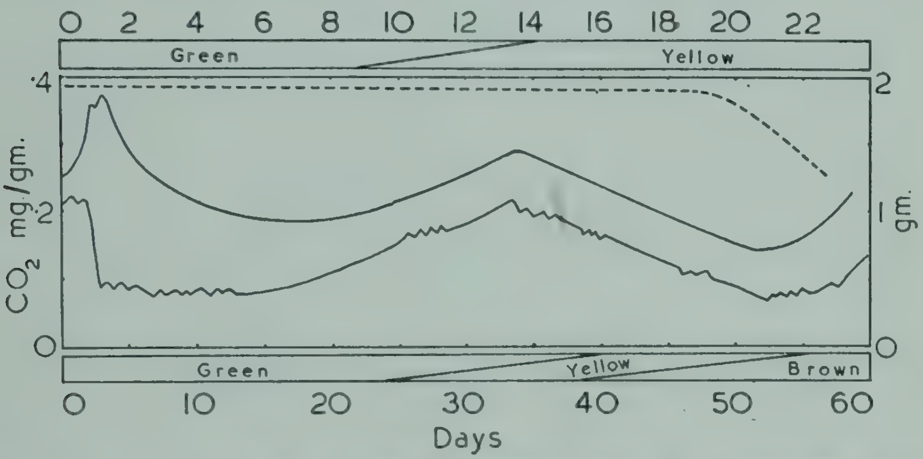

FIG. 7. Respiration drifts of detached cherry-laurel and *Tropaeolum* leaves. Bottom curve and time scale refer to cherry-laurel; upper solid curve and time scale to *Tropaeolum*. The broken line gives the leaf weight of *Tropaeolum* with ordinates on the right.

The drift curves of barley leaves have also been studied in detail. Individual curves show much variation; but also constant features that afford interesting comparisons with cherry-laurel. In Fig. 8, a generalized barley curve, based on numerous experiments in the Oxford laboratory, is plotted with one for cherry-laurel at an approximate temperature of 20° C. The time scales are very different. The drift that may occupy over a month in cherry-laurel is completed in about six days by barley. Conversely, the initial rate of cherry-laurel is about one-fifth that of barley and, in spite of fluctuations, a wide difference of rate is maintained. Nevertheless, by suitably adjusting the time scales, certain similarities of form, and also some important differences, are easy to see. Phases 1 and 2 occur in both; but the phase 2 of barley is interrupted at a relatively high level and no low, steady phase 3 is observable. It is, in fact, not always evident with cherry-laurel either at the higher temperature. Phases 4 and 5 are represented in the barley curve by a high undulating plateau on which a rise (phase 4) and fall (phase 5) round a single peak are not clearly defined. There is usually a small rise after 4–5 days (phase 6) followed by a sudden plunge towards extinction. Curves for wheat published by Krotkov (1939) show phases

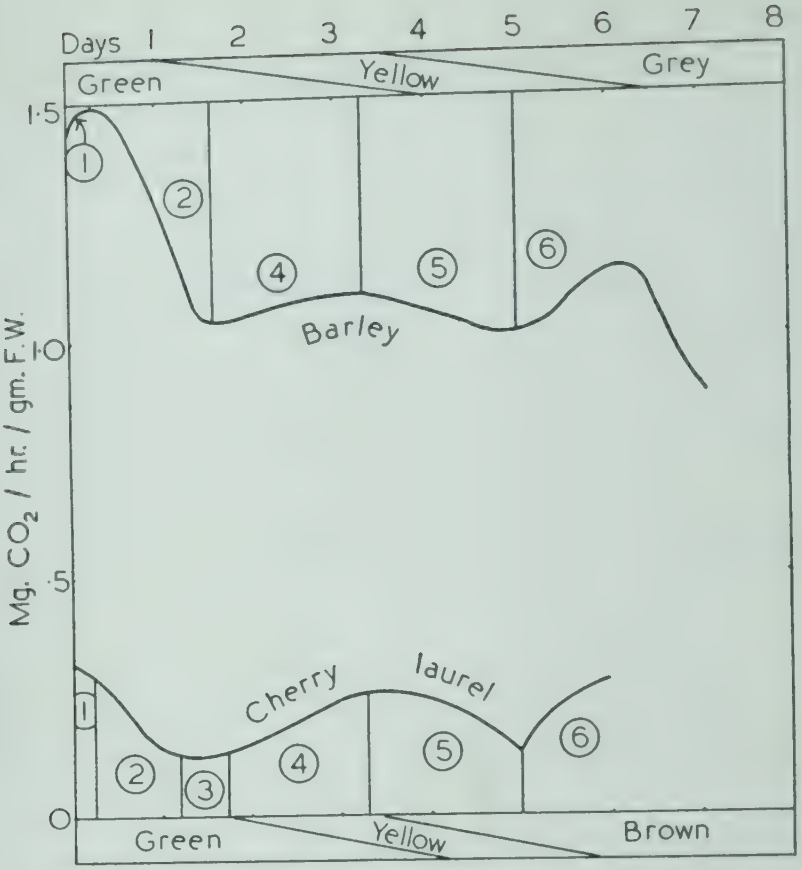

FIG. 8. Comparison of detached barley and cherry-laurel leaf drift-curves.

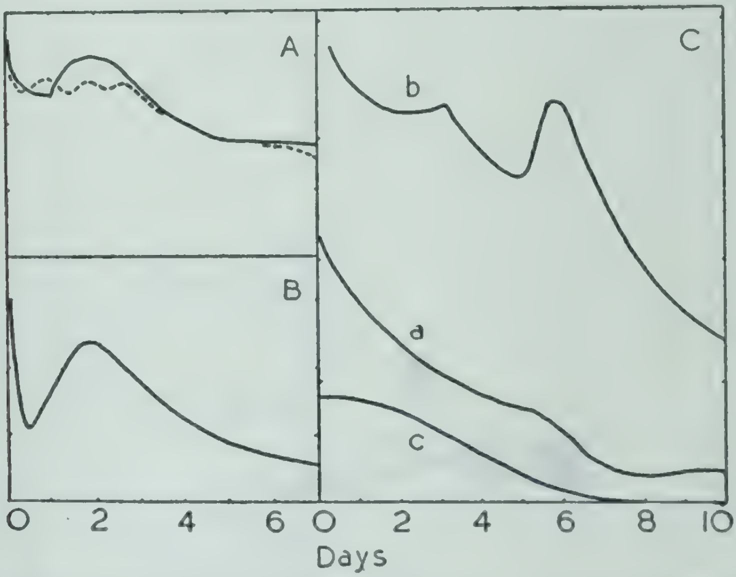

FIG. 9. CO_2-drift curves. A, Sudan grass (two curves) and B, Kikuyu grass (Wood, Cruickshank, and Kuchel, 1943). C, barley leaves from different positions of insertion on a mature stem. *a*, top leaf still unrolling; *b*, second leaf, lamina fully spread; *c*, old leaf towards base of stem, losing colour. Author's data. CO_2-scales omitted for clarity.

2 and 4 well developed and the early stages of phase 5. Curves for Kikuyu (*Pennisetum clandestinum* Hockst.) and Sudan grass (*Andropogon sudanensis* L. and B.), also members of the same family, have been published by Wood *et al.*[1] They show obvious resemblances with those for barley, and occupy about the same time, 6 or 7 days. Phase 1 does not appear, a sharp fall (phase 2) starting immediately. This is interrupted, usually at a high level, after about one day, by a rise (phase 4), followed by a fall (phase 5). In some experiments with Sudan grass the regular rise and fall is replaced by an undulating plateau, as in barley (Fig. 9A, dotted curve). There is usually no sharp rise (phase 6) due to saprophytes. Perhaps these investigators were more successful than others in maintaining aseptic conditions. The analytical results provided also suggest that little respirable material was left in the leaves at the breakdown stage. Diametrically opposed results were shown by broad-bean leaves (Yemm, 1934) in which a prolonged and more or less continuous fall was interrupted on the fifth day by a massive rise associated with the development of saprophytes. This effect was even more pronounced in leaves suffering potassium starvation (Fig. 10). Succulent leaves of *Kleinia articulata* (Thoday and Richards, 1944) show a CO_2-drift very reminiscent of cherry-laurel and extending over 11 days. In well nourished leaves phases 1 and 2 are represented by two days showing a regular rhythm superimposed on the gradual fall. Phases 4 and 5 are represented by a well-developed single hump, and there is a later sharp rise (phase 6) associated with cell breakdown.

When these various curves are compared, the initial choice of cherry-laurel is seen to have been singularly fortunate because its stately progression of events allows scope for the full development of each successive stage. Species such as *Tropaeolum* and *Kleinia* may show the same stages with almost equal clarity; others, like barley and Sudan grass, may be referred to the same sequence supposing some stages to overlap and others to be telescoped.

Colour changes

Visible alterations accompany the CO_2-drift curve in fairly close correlation. During phases 1 and 2 little is noticeable; but by the beginning of the third phase there is a definite loss of pigment. The green colour becomes pale and begins to be replaced by yellow—except, apparently, in *Kleinia*. After the crest is passed, cherry-laurel leaves begin to turn brown, due to cellular oxidations of polyphenols. Barley and similar species without polyphenolases do not show this coloration; but the leaves become translucent and grey owing to the sap escaping from collapsing cells into the intercellular spaces. At the same time a marked loss of fresh weight (Fig. 7) is caused by exudation from the surface.

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[1] Wood, Cruickshank, and Kuchel, 1943; Wood, Mercer, and Pedlow, 1944.

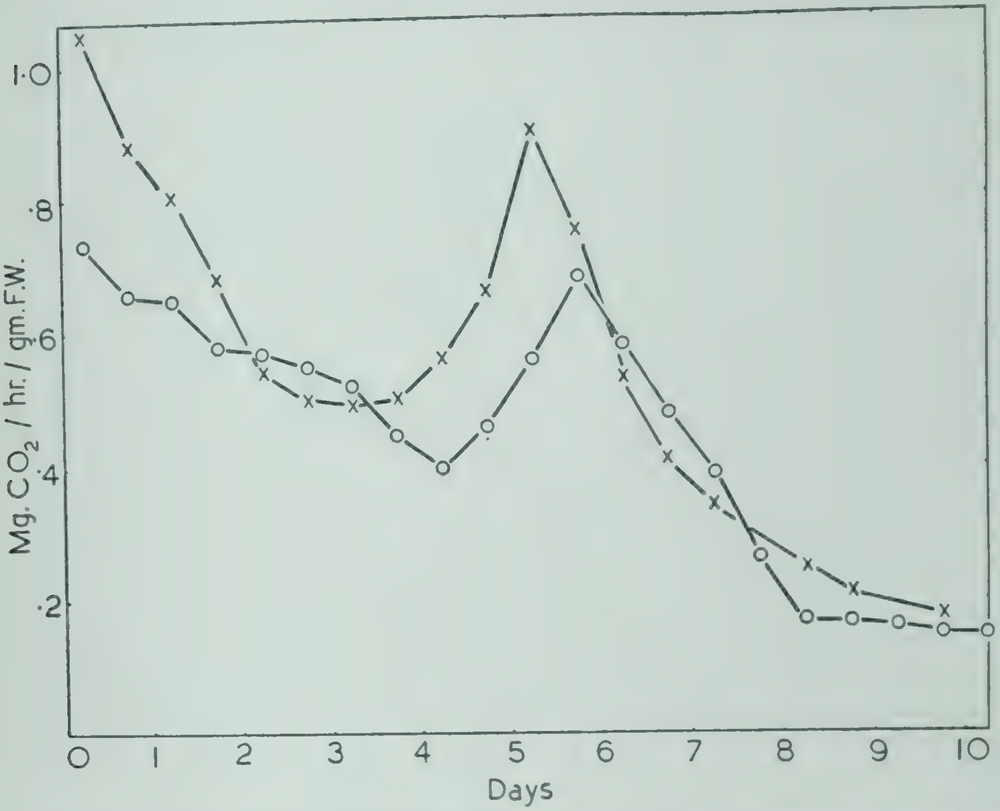

FIG. 10. CO_2-emission of broad-bean leaves (third from top) from plants raised in water culture; ○ with full mineral nutrient solution; × deficient in potassium. After Yemm, 1934.

Age

So far, description has been limited to completely expanded leaves still in full vigour and well nourished; picked, that is to say, after a period of active photosynthesis. The course of the curve depends greatly on developmental stage. In young leaves the respiratory hump or high plateau is much reduced and in very young ones may be altogether lacking. Fig. 9C represents the behaviour of leaves plucked from barley stems at three levels. Young leaves still unrolling (*a*) showed only a continuous fall to a slow rate. Leaves with their laminae recently spread (*b*) showed an interrupted fall followed by a saprophytic rise on the sixth day, and senescent leaves, already losing colour when plucked, showed a slow initial respiration that steadily declined (*c*). Young cherry-laurel leaves also showed a sharp decline to a low value with a late and much reduced rise associated with yellowing.[1]

---

[1] Godwin and Bishop, 1927.

Nature of the successive phases

Phase 1, immediately after detachment, corresponds more or less with the normal respiration of the leaf *in situ*. It is liable to disturbances caused by change of temperature, nearly always a rise, in passing from the open air to the experimental chamber; and by stimulation due to handling.[1] There is usually an excess of carbohydrate in the leaf, mainly starch, for example, in cherry-laurel, sucrose in barley and Sudan grass, and mixed sugars in Kikuyu. The respiration quotient (R.Q.) keeps close to unity,[2] suggesting a predominant consumption of carbohydrates. Barley leaves with a low content of available carbohydrate when cut showed an initial R.Q. = 0·88 and neither phase 1 nor phase 2 was visible in the CO_2-drift curve (Fig. 11).

Direct analysis of total carbohydrate loss presents many technical difficulties; but has been achieved with several species. The classic investigation is that of Deleano (1912) with vine leaves which at 19° C. have a long-drawn-out drift of about 20 days. He found (Fig. 12) that for the first hundred odd hours the CO_2-output could be accounted for by loss of carbohydrate, mostly starch; proteins showed no breakdown during this period (Fig. 12). More recently, with improved methods, Yemm has analysed the available carbohydrates in broad bean and barley leaves. Available carbohydrates are taken to mean the sugars and those polysaccharides for whose hydrolysis enzymes are available. They here include starch, fructosans, sucrose, glucose, and fructose, but not cellulose. Table 11 marshalls Yemm's data for the first 6–12 hours. In beans the carbohydrate loss fully accounted for the CO_2 produced,

TABLE 11

CO_2-output and CO_2-equivalent of carbohydrate loss in mg./hr./gm. fresh weight

Data of Yemm, 1934

Leaf	*Hours*	*CO_2-output*	*Carbohydrate loss*	*CO_2 unaccounted for*
Broad bean	12	0·61	0·62	. .
Barley	12	1·50	1·57	. .
,,	12	1·45	1·10	0·35
,,	12	1·26	0·89	0·37
,,	6	1·39	1·50	. .
,, mean	. .	1·40	1·27	0·13
,, senescent leaves	12	1·01	0·44	0·57

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[1] See Audus, 1935; Godwin, 1935; Barker, 1935.

[2] Yemm, 1934; McKee, 1937*a*.

and in barley the mean discrepancy was less than 10 per cent. Senescent leaves showing no phase 1 (cf. Fig. 9*cc*) had a large excess of CO_2, not of carbohydrate origin, from the start. Corresponding results for wheat by Krotkov indicated that loss of carbohydrates, especially sucrose, accounted for the CO_2-output of the first 24 hours, a period which appeared to cover both phase 1 and phase 2. Krotkov's results are summarized in Table 12. The grasses examined by Wood *et al.* showed an excess of CO_2-production over carbohydrate loss even on the first day. The authors remark that their carbohydrate fractions were not exhaustive. Further, the CO_2-drift curves show no phase 1, and shorter periods, such as those employed by Yemm with barley, might

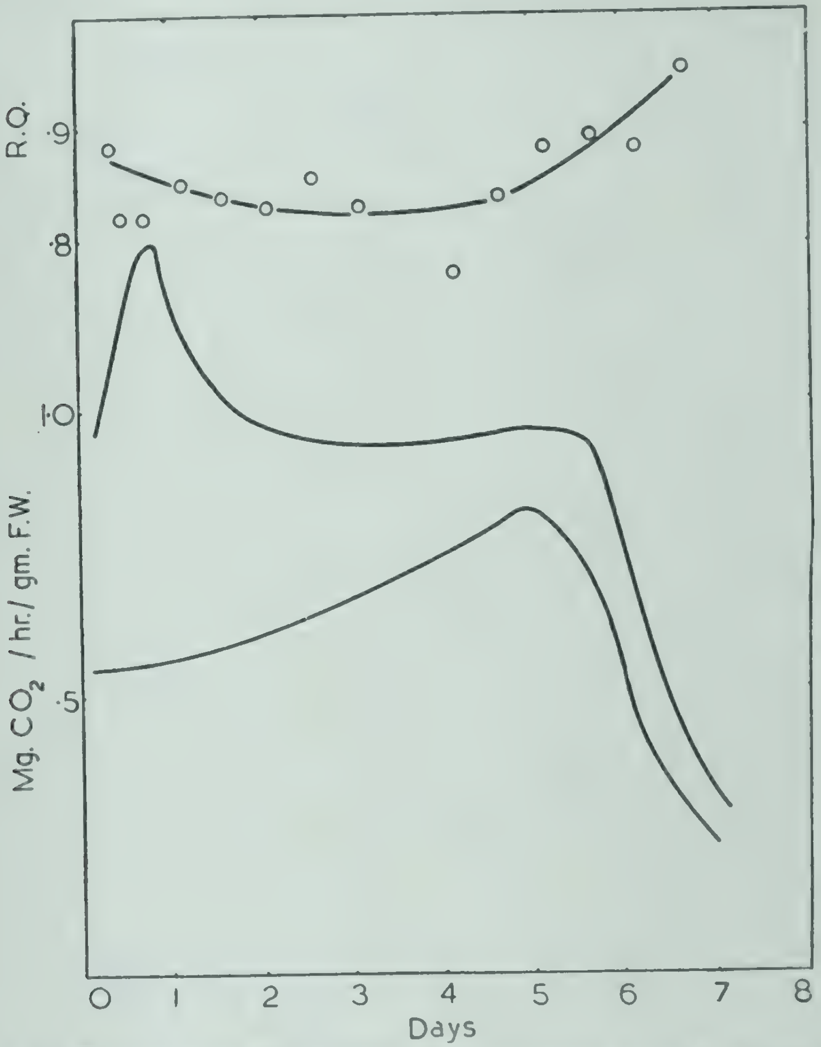

FIG. 11. Bottom curve, CO_2-emission of barley leaves cut after a period of darkening on the plant. Middle curve, CO_2-emission of leaves cut simultaneously but after a period of illumination. Top curve, R.Q. of leaves darkened before cutting. After Yemm, 1934.

have told a different tale. The same remark may also be applied to the rhubarb-leaf data of Vickery and Pucher (1939).

Phase 1 and its transition to the early part of phase 2 indicate what goes on in the leaf while still attached to the plant. It is a phase in which available carbohydrates provide the respirable material, but this is not the same thing as saying that carbohydrates are always and only the substrate of normal plant respiration. Not all leaves show

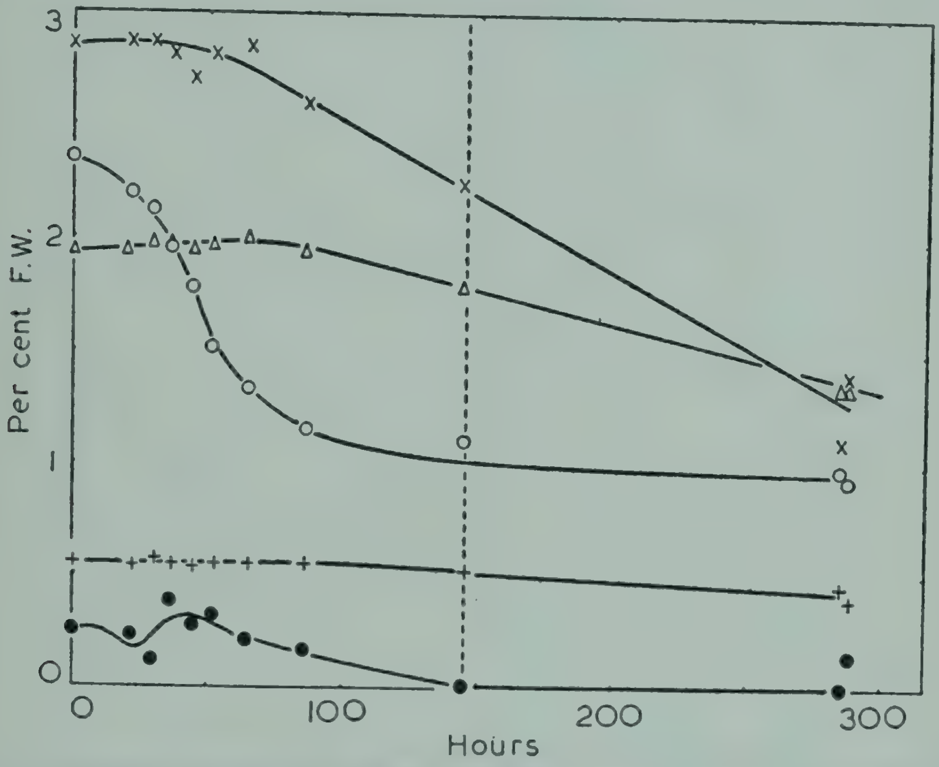

FIG. 12. Analyses of halved vine leaves, kept in the dark. ○, starch; ●, sucrose; ×, hexose; △, total acid calculated as tartaric; +, coagulable protein. The persistent 'starch' fraction after 144 hours gave no iodine colour reaction. Up to this time (indicated by a broken vertical line) total carbohydrate loss was equivalent to CO_2-output. Data of Deleano, 1912, adjusted to allow for variations in control half-leaves.

TABLE 12

CO_2-*output and* CO_2-*equivalent of carbohydrate loss by wheat leaves in mg./gm. F.W.*

Data of Krotkov, 1939

Day	1	2	3	4	5
Phase	1–2	3	3	4	4
CO_2-output	6·89	7·26	10·09	10·08	5·09
CO_2-equivalent of carbohydrate loss	7·05	2·79	1·91	1·61	0·95
$100 \times \frac{\text{carbohydrate } CO_2}{CO_2\text{-output}}$ . .	102	38	19	16	19

phase 1, which is typical of juvenile to mature leaves in a high state of carbohydrate nutrition. Very young leaves, senescent leaves, leaves of plants forced in greenhouses, or picked with a low carbohydrate content after darkening, show an immediate and usually rapid decline of CO_2-emission.

Phase 2 is shown to a greater or lesser extent by almost all leaves. Its rapid decline is associated with a progressive exhaustion of the available carbohydrates, particularly sucrose and the polysaccharides. Relations between individual carbohydrates are complex and are gone into more fully on p. 102. All appear to be readily inter-convertible, and for present purposes it is enough to regard the total drain upon the pool. By the end of phase 2 the carbohydrates have been much reduced, but are not exhausted. As it proceeds, there is a progressive increase of carbon dioxide not accounted for by carbohydrate loss; and at the same time a progressive decline of the respiratory quotient from 1·0 to 0·8, a further indication that some other class of substrates is being called upon. Detailed results have been provided for barley and broad beans by Yemm (Figs. 13, 35, and 36).

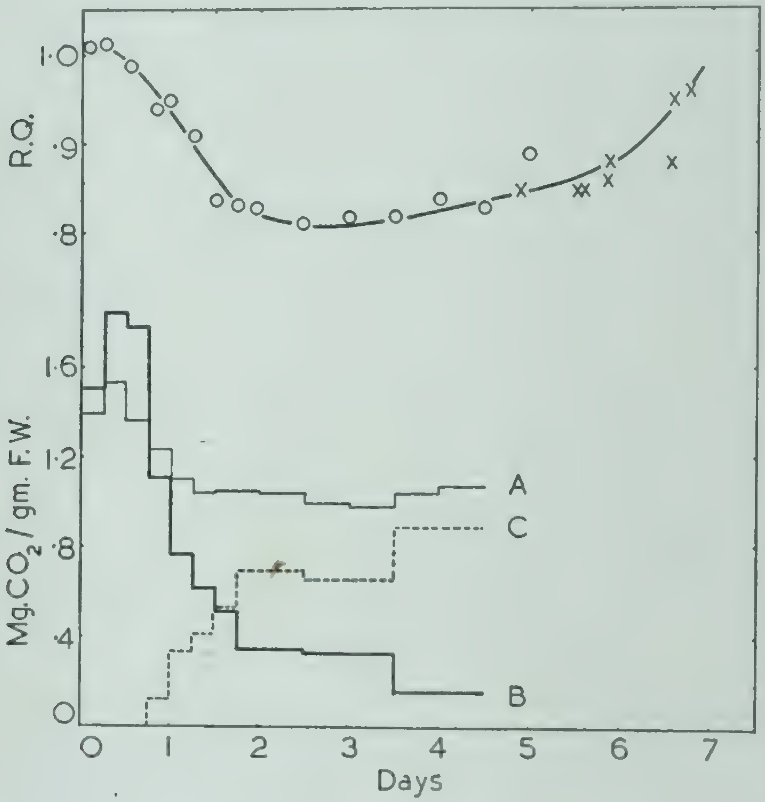

FIG. 13. Cut barley leaves. Curve A, CO_2-emission; curve B, CO_2-equivalent of carbohydrate loss; curve C, CO_2 unaccounted for. Top curve, respiratory quotient. The continuation of the R.Q. curve is added from a second experiment. After Yemm, 1936.

The end of phase 2 is reached when the CO_2-emission curve no longer falls, and this may occur at either low or high level. If the rate at the end of phase 2 (r_2) is related to the initial rate (r_1) the values of r_2/r_1 obtained with different species fall into a more or less continuous series. Leaves with long drifts have low r_2/r_1 values and short-drift leaves have high ones (Table 13).

TABLE 13

Characteristics of CO_2-drift curves of leaves of various species

	Temp. °C.	CO_2-*output (mg.* CO_2*/gm. F.W./hr.)*		r_2/r_1	*Duration of drift in days*
		r_1	r_2		
Cherry-laurel (Blackman)	16·5	0·21	0·07	0·33	54
„ „ (Godwin and Bishop) . . .	20·8	0·30	0·13	0·43	20
Kleinia articulata . .	25·0	0·09	0·04	0·45	9
Tropaeolum majus . .	16·5	0·37	0·19	0·51	21
Kikuyu grass . . .	24·5	0·27	0·15	0·56	5
Sudan grass . . .	24·5	0·28	0·17	0·61	?
Wheat	20·0	0·31	0·20	0·65	5
Barley (Yemm) . .	25·0	1·5	1·00	0·67	6
„ (McKee) . .	21·0	1·2	0·9	0·75	6
Broad bean . . .	25·0	0·65	0·59	0·91	4

This might be taken to indicate that long-drift leaves reduced their carbohydrate content to a lower level than short-drift leaves before utilization of secondary substrates became considerable. Direct analytical data do not bear this out; the ratio of available carbohydrates at the end of phase 2 to the initial is about 1/2 in the most diverse leaves; and the concentration relative to fresh weight is lower in short-lived leaves than in long (Table 14).

TABLE 14

Available carbohydrate in long- and short-drift leaves at the end of phase 2

	Average drift in days	*Available carbohydrate**		
		Per cent. F.W.	*Per cent. initial*	
Cherry-laurel . .	20	2·47	49·1	Onslow, unpublished
Vine	20	4·28	59·2	Deleano, 1912
Barley	6	1·79	49·7†	Yemm, 1934
Wheat	5	0·8–0·15	61·5–26·7	Krotkov, 1939

* Calculated as hexose equivalent. † Mean of 4.

Phase 3, the period of slow CO_2-evolution at a steady level, is not often revealed, because the rise of phase 4 anticipates its occurrence. It probably has analogues in the ground respiration of partially starved wheat roots[1] and the endogenous respiration of unicellular algae.[2] Both the last are highly resistant to poisoning by cyanide, unlike the floating respiration induced by addition of sugars. The effect of cyanide on phase 3 respiration of cherry-laurel does not seem to have been examined; but these examples agree in having a slow rate associated with a low content of available carbohydrate.

Phase 4. Whatever the duration of the drift, the carbohydrates now make a progressively smaller contribution to the CO_2-output. The first attempt to identify a secondary substrate appears to have been that of Godwin and Bishop (1927), using cherry-laurel. They observed that the cyanogenetic glycoside (prulaurasin) was stable for about 4 days, i.e. until towards the end of phase 2; but that rapid loss of HCN occurred during the early stages of phase 4. The glucose released by the hydrolysis of the glycoside was naturally regarded as available for respiration and as contributing to the secondary rise of respiration rate. A temporary increase of hexose concentration occurred at this point, while sucrose and starch continued to diminish steadily.[3] In young leaves with delayed phase 4, glycoside hydrolysis was delayed correspondingly.

This obviously represents a rather special case, and the CO_2-equivalent of the glycoside lost was only a small fraction of the additional CO_2-emission (Fig. 14). A significant observation later made by Yemm (1934) and McKee (1937*a*) with barley was the lowering of the R.Q. which sank to about 0·82 when phase 4 was well advanced (Fig. 13). The only possible cause for such a fall appeared to be the massive breakdown of proteins. Organic acids would tend to raise the R.Q. if respired, and were not believed to be present in sufficient quantities to contribute much carbon dioxide. This was later verified by Somers.[4]

The behaviour of proteins and their products in detached leaves has been extensively studied.[5] The time at which protein breakdown becomes detectable at 20–25° C. varies from less than a day in the grasses to about 5 days for vine. It anticipates visible yellowing, breakdown of the chlorophyll, and lowering of the R.Q. It appears to occur, at least in some leaves, while the carbohydrate loss is still adequate to account for total CO_2-production.

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[1] Lundegårdh, 1949.

[2] Emerson, 1927.

[3] Analyses carried out for the authors by Mrs. Onslow.

[4] Somers, unpublished.

[5] In vine, Deleano, 1912; barley, McKee, 1937*a*, 1950, Yemm, 1937; tobacco, Mothes, 1931, Vickery, Pucher, Wakeman, and Leavenworth, 1937; *Tropaeolum*, Michael, 1935; broad bean, Mothes, 1925; runner bean, Mothes, 1925; Sudan grass, Wood, Cruickshank, Kuchel, 1943; Kikuyu grass, ibid.; oat, Cruickshank and Wood, 1945; belladonna, James, 1950*b*.

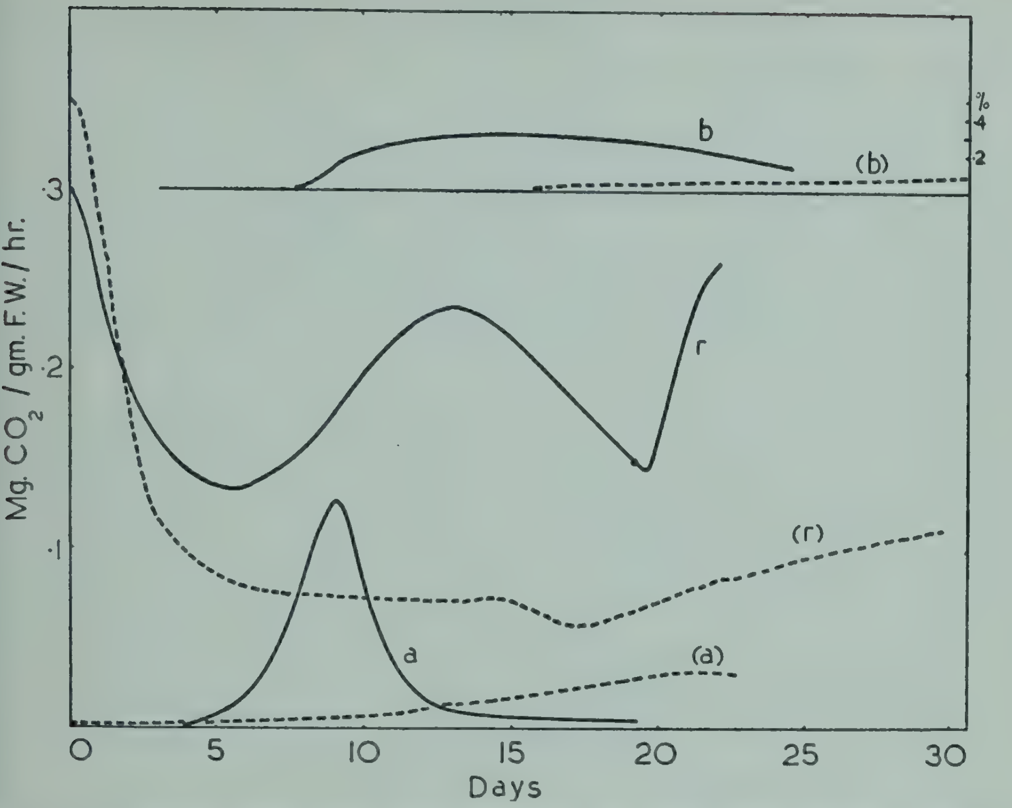

FIG. 14. CO$_2$-emission and loss of HCN-glycosides from cherry-laurel leaves. *a* and (*a*) loss of glycoside as CO$_2$-equivalent; *b* and (*b*) rate of yellowing as percentage of leaf surface per hour; *r* and (*r*) rate of CO$_2$-emission. Continuous lines—leaves 21 months old; broken lines—leaves $2\frac{1}{2}$ months old. After Godwin and Bishop, 1927.

TABLE 15

	Time proteins stable (days)	*Length of drift (days)*
Vine	5	20·5
Belladonna . . .	2	7
Barley	< 1	6
Oats	< 1	4
Sudan grass . . .	< 1	?
Kikuyu grass . . .	< 1	5

The changes resulting from protein breakdown are numerous and complex and have been most closely related to the respiratory sequence in barley.[1] In mature leaves, protein breakdown is already apparent after 6 hours isolation, and may even be happening in attached leaves as part of a translocation mechanism. Such hydrolyses do not necessarily lead on to a complete respiratory breakdown of the nitrogenous materials. During the first 24 hours, the CO$_2$ produced by barley leaves

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[1] Yemm, 1937.

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appears to come wholly, or almost wholly, from the available carbohydrates. During this period the loss of protein is accounted for by accumulation of amino-acids, glutamine, and other soluble organic nitrogen compounds such as peptides. There is no accumulation or release of ammonia. The glutamine, however, accumulates in excess of the amount that could have been preformed in the lost protein; accounting for about 4 times its whole loss of amide-N.[1] It is therefore possible that some protein is fully decomposed to provide ammonia for the additional amide groups, and that a small CO_2-contribution might result.

Much more definite changes were observed after 48 hours, i.e. when the leaves had passed from phase 2 into phase 4. The accumulation of glutamine continued, asparagine was formed rapidly, implying a corresponding formation and fixation of ammonia, and there was even a release of some free ammonia (Fig. 15). At the same time, amino-N began to diminish,[2] and the conclusion seems inescapable that complete protein degradation was occurring with the formation of the two final products, ammonia and carbon dioxide.

The breakdown of protein was much slower in young leaves, and the glutamine formed could have arisen directly, in agreement with the absence or postponement of phase 4 previously noted (p. 50) for young leaves.

TABLE 16

Barley leaves. Results given in mg. N *per gm. fresh weight*

Data of Yemm[1]

Period of isolation (hr.)	*Loss of protein* N		*Amide* N *in lost protein*		*Increase of glutamine* N	
	Young	*Mature*	*Young*	*Mature*	*Young*	*Mature*
24	1·17	0·94	0·060	0·09	0·060	0·42
48	2·26	2·34	0·115	0·23	0·125	0·84
72	. .	2·60	0·250	0·26	0·250	0·95

Similar results are available for mature leaves of oats, Sudan and Kikuyu grasses.[3] All show early loss of protein with accumulation of amino-nitrogen. Glutamine formation is much less than in barley, but asparagine accumulates rapidly after 48 hours; i.e. with the beginning of phase 4. Free ammonia is released later. At the same time, i.e. towards the end of phase 4, amino-N begins to be reduced. The individual amino-acids contributing to it have been to some extent

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[1] Data of Yemm in Chibnall, 1939, p. 219.

[2] Amino-N exclusive of that in amides (glutamine and asparagine); probably mainly in amino-acids.

[3] Wood and collaborators, loc. cit.

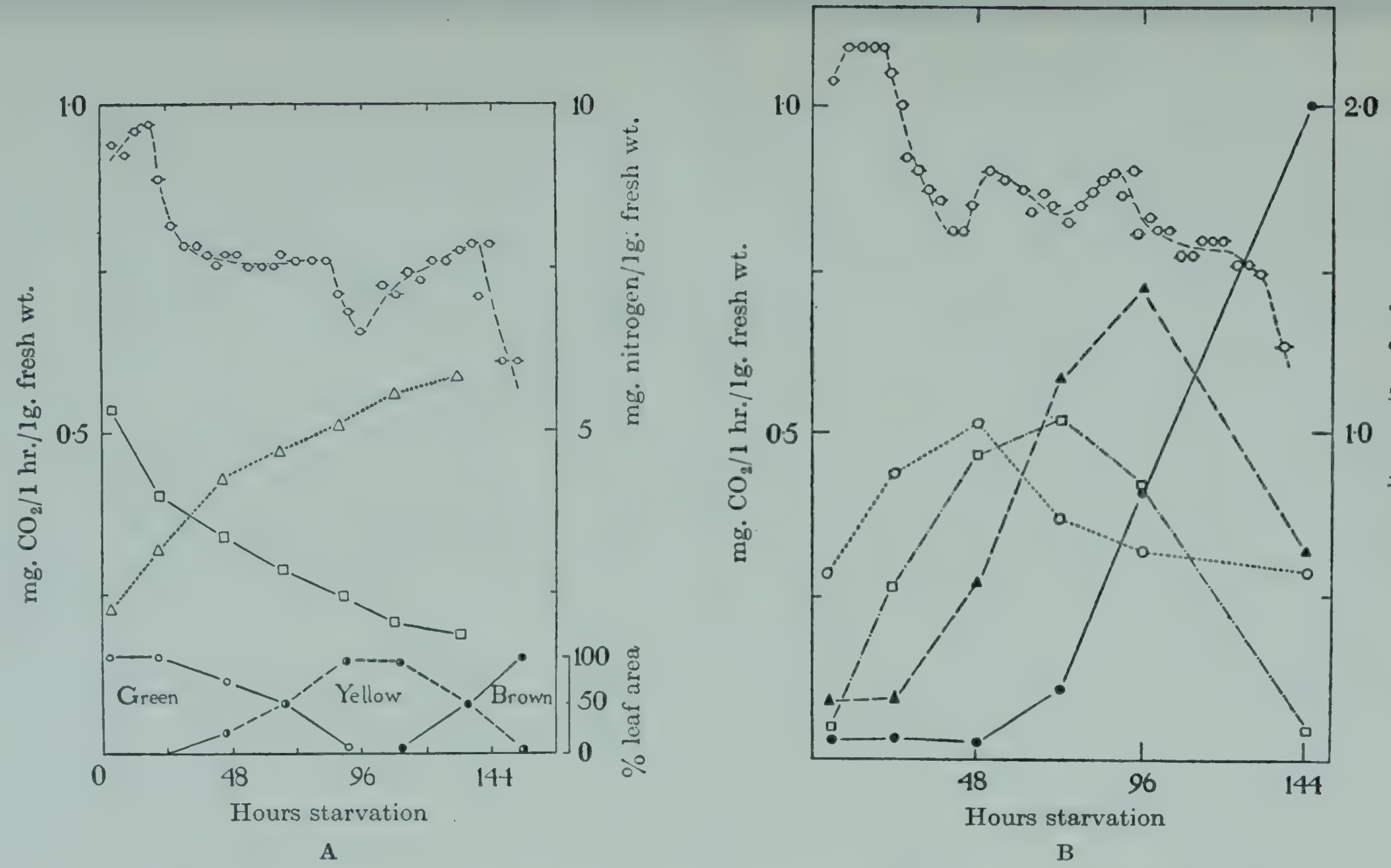

FIG. 15. Changes of soluble nitrogen fractions in detached barley leaves. A. –○– CO$_2$-emission; –□– total insoluble-N; ···△··· total soluble-N. Percentage of area green, &c., shown below. B. ···○··· amino-N; –·–□–·– glutamine-N; – – ▲– – asparagine-N; —●— ammonia-N. The CO$_2$-emission curve –○– is given above. From Yemm, 1937.

particularized; glutamic acid and cystine appear to break down relatively fast, tyrosine and tryptophan more slowly.[1]

It is not so easy to calculate the amount of CO_2 derivable from the protein losses as from the carbohydrate, on account of the more numerous and dubious changes occurring. The Kikuyu results are relatively simple in that the sum of protein+amino+amide+ammonia nitrogens remained approximately constant over the whole drift period. It may therefore be assumed that the changes among them are self-contained, and that no carbon passes between them and other substances except CO_2. There is no measurable formation of glutamine or unidentified N-compounds. Wood, Mercer, and Pedlow propose the following conventions: Protein-C and amino-acid-C = 3×protein-N and 3×amino-N respectively; asparagine-C = 3·43×amide-N. The amount of protein-C disappearing and not accounted for by asparagine formation is given by the algebraic sum of the C-changes of protein, amino-acids, and asparagine. It is presumed that this is all oxidized to CO_2. Results of two experiments calculated on these assumptions are given in Fig. 16. The degradation of protein is seen to give rise to a progressively larger proportion of the total CO_2-output, and to account for most of it towards the end of the drift.

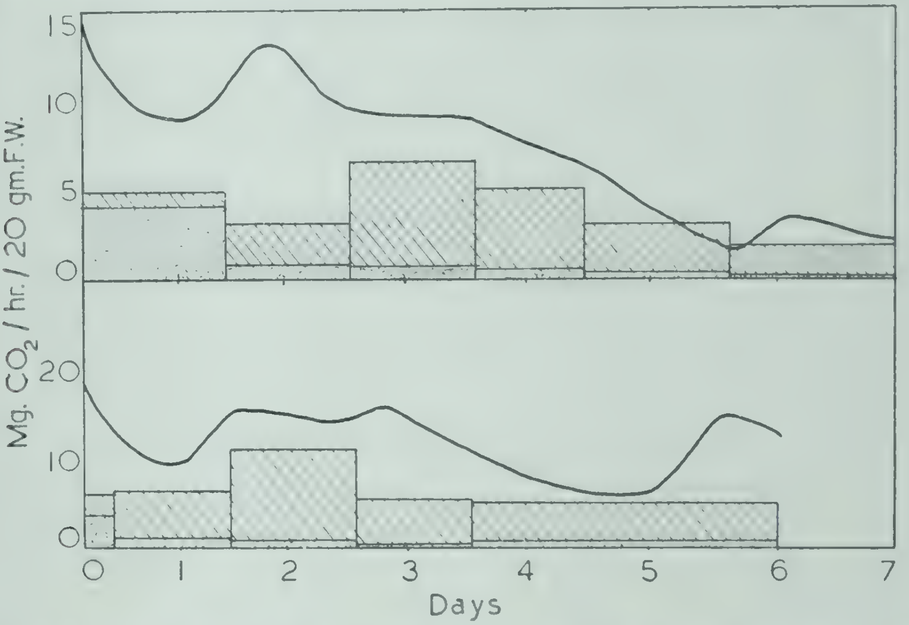

FIG. 16. Kikuyu grass leaves. CO_2-emission is shown by the continuous curves. The CO_2-equivalent of carbohydrate lost is shown by dot shading and the CO_2-equivalent of protein loss by line shading. Two separate experiments are represented. From Wood, Mercer, and Pedlow, 1943.

The estimation is rather more complex for barley on account of the accumulation of glutamine and unidentified N-compounds to a considerable amount. To make the calculation, Yemm (1950) uses the arbitrary assumption that the carbon of the unidentified N-compounds weighed twice as much as the nitrogen; and further that glutamine-C = 2·14 × glutamine-N (= 4·28 × unstable amide-N), asparagine-C = 1·71 × asparagine-N (3·42 × stable amide-N), protein-C = 3·1 × protein-N, and amino-C = 2·8 × amino-N. On this basis, he found that, by the end of the drift in eight experiments, the C-loss from protein sources had accounted for between 20 per cent. to 40 per cent. of the total CO_2 emitted. In three experiments in which the carbohydrates were determined, their total loss of carbon was equivalent to 35, 47, and 29 per cent. of the total CO_2-emission respectively. In one experiment in which the leaves started with an exceptionally high carbohydrate content, the proportion rose to 71 per cent. Apart from this one experiment there appears to be a substantial amount—some 20–40 per cent.—of the CO_2 whose origin is not accounted for in either carbohydrate or protein loss. A similar discrepancy is noticeable in Fig. 16. According to available data this cannot come from organic acids such as malic and citric.[1]

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[1] Wood and Cruickshank, 1944.

Visible yellowing of the leaves is also a characteristic of phase 4. It begins usually in the early stages and is more than half completed by the end (Figs. 7 and 8). It is due to breakdown of the chlorophylls which, according to Michael, is measurable before yellowing can be seen. The breakdown of protein may begin earlier than either. In mature barley leaves, proteins begin to disappear within the first 6 hours, chlorophyll losses become detectable after about 24 hours, and visible yellowing after about 48 hours. In *Tropaeolum* leaves[2] protein loss is more rapid than chlorophyll loss for 2 or 3 days, and thereafter runs a more or less parallel course. Yellowing is first visible after 5 days. If protein breakdown is delayed, chlorophyll breakdown is delayed also.[3]

Extracted chlorophylls are unstable in light and air and probably owe their stability *in vivo* to a chlorophyll-protein linkage. Phyllochlorin, defined by Mestre to include 'the green pigments of the plastid', is likely to consist of a chromoprotein in which the porphyrin of chlorophyll is weakly linked to protein through the magnesium atom. Although a pure chlorophyll-protein has not yet been prepared, there is considerable evidence for the existence of such substances in leaves.[4] The breakdown of the proteins within the grana of the chloroplasts may therefore unmask the chlorophyll, and make it available to respiratory breakdown. Attempts have been made to discover whether the chloroplast proteins are more or less stable than the cytoplasmic. Chibnall and Grover (1926) found that the ratio of chloroplast protein N/cytoplast protein N had not changed in *Phaseolus multiflorus* leaves after 4 days isolation. Chloroplast and cytoplasmic proteins were also found to break down at similar rates in Kikuyu grass (Wood *et al.*, loc. cit.), but in Sudan grass leaves chloroplast proteins were lost the faster.

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[1] Somers, unpublished; Wood, Cruickshank, and Kuchel, 1943.

[2] Michael, 1935.

[3] Wood, Cruickshank, and Kuchel, 1943.

[4] Lubimenko, 1921, 1927; Noack, 1927. See Rabinowitch, 1945, for discussion.

Phase 5. The transition from phase 4 to phase 5 is well defined in leaves such as cherry laurel with a sharply peaked respiration hump (Fig. 7). In others, where the peak is replaced by a high plateau (Figs. 8 and 9), no sharp division shows in the CO_2 drift curve. Nevertheless, the end of the plateau corresponds with a fundamentally different condition from that of its beginning, as is indicated by the drifts of the various N-fractions (Fig. 15). These come to successive maxima in the order amino-acids, glutamine, asparagine, representing so many successive stages in the consumption of the proteins. As each in turn begins to disappear, a further step has been taken towards exhaustion. In the type of curve with a single peak, the secondary substrates may be supposed to consist mainly of one, or to become available more or less in unison; when it is replaced by a rolling plateau, the substrates perhaps become available in successive waves.

The proteins are not completely removed when phase 5 comes to its end, and usually they then appear to be approaching a steady value at a fairly high level (Fig. 15A). One possible reason might be that the tissues contain a proportion of non-digestible, or at least highly resistant, protein; another is mentioned on the next page in connexion with phase 6.

During phase 5 the R.Q., which has dropped to about 0·8 in phase 4, begins to rise again (Fig. 13). This would happen if there were a considerable consumption of organic acids at this late stage, stated by Moyse to occur in leaves of *Rumex acetosa* which have a relatively high content of oxalic acid. A more usual reason for the rise lies in the changed nature of the protein breakdown. While the formation of amides, associated with incomplete oxidation, leads to R.Q.s in the neighbourhood of 0·7, the further oxidation of amides and amino-acids to carbon dioxide and ammonia gives R.Q.s in the range of 1·0 to 1·3. It is this latter change that predominates in phase 5 (Fig. 15 and Table 21). Similar results were obtained by Moyse with wheat and *Polygonum fagopyrum*.

Nevertheless, loss of these substances did not wholly account for the CO_2 output of Kikuyu grass (Fig. 16). To fill the gap it is possible that hitherto unavailable carbohydrate might begin to break down. Support for this idea is given by Buston's analyses of hemicelluloses in detached leaves of vine, runner beans, and maize. After prolonged periods of isolation, although there was no loss of pectin and insoluble hemicelluloses, he found considerable breakdown of hemicelluloses that could be made water-soluble by treatment with caustic soda. These consisted largely of pentosans, uronic anhydrides, and some hexans. In maize the loss amounted to 19 per cent. of their initial weight which accounted for 14·7 per cent. of the total dry matter of the leaves. The R.Q. to be expected by the complete oxidation of such substances to CO_2 would approximate unity, and so tend to raise the observed R.Q. above the earlier values around 0·8. There is no indication from Buston's results at what stage the breakdown of the less resistant hemicelluloses is fastest. It seems plausible to suppose that the incipient degradation of protein structure during phase 5 allows hydrolysing enzymes to approach the hemicelluloses previously inaccessible at their location in the cell wall.

Phase 5 is terminated by the collapse of cell organization, perhaps brought on by the rapidly increasing amounts of ammonia liberated at this stage. Feeding belladonna leaves with heavy doses of ammonium sulphate or arginine, from which ammonia is released, brings on the collapse at an earlier stage than feeding with other amino-acids.

Phase 6. So far as the individual cell is concerned, the end comes somewhat suddenly The protoplasmic organization breaks down so that semipermeability is lost and the vacuole's contents ooze into the intercellular spaces and even on to the leaf surfaces. Taking the complex organ as a whole, this end point is somewhat slurred by the idiosyncrasies of cell tempo. Owing to the progressive injection of its intercellular spaces, the leaf becomes translucent and greyish and at the same time there is a pronounced loss of weight (Figs. 7 and 8). Autolysis, i.e. unorganized enzyme catalysis, accelerates numerous reactions previously in abeyance, such as the oxidation of polyphenols in tea, and the hydrolysis of tropane alkaloids in belladonna. The irreversible oxidation of polyphenols as, for example, in leaves of cherry-laurel, belladonna, and beans, causes browning of the tissues; in other species there is no marked colour change. It is remarkable that while the general effect of autolysis is to accelerate the breakdown of the cell substances remaining, loss of proteins comes almost to a standstill (Fig. 15A). This is perhaps explained by an inactivation of proteases outside the living structure of the cell. Barley leaves, disintegrated immediately after detachment, show a slower rate of protein hydrolysis than whole leaves (Fig. 17), and at the same time the protective effect of the proteins on chlorophyll is maintained. The author found that mushes of fresh barley leaves kept under toluene were still bright green after 5 days when normal leaves are completely yellow.

It is not known how much of the rising CO_2-output of phase 6 is due to the leaf's own enzymes; the great bulk of it comes from the respiration of saprophytic moulds and bacteria, which at this stage, if not before, gain access to the cell contents.

Causes of the drift

A drift of respiration rates happens in all tissues and those of detached leaves may be regarded to a very large degree as accelerations of the drifts that would have occurred had the leaves been left undisturbed on the plant (Fig. 29). Their yellowing and exhaustion have something in common with the normal condition of leaves in autumn (cf. p. 80). For either drift, an explanation involves both depletion of substrates and changes of protoplasmic condition. It is easy to appreciate that the isolation of darkened leaves will accelerate starvation; but it commonly also leads to faster degradation of their protoplasts. The distinction between plastic and protoplasmic proteins in leaves is vague, even supposing it to exist; detachment seems to make at least a part of the structural protein more liable to early respiratory breakdown.

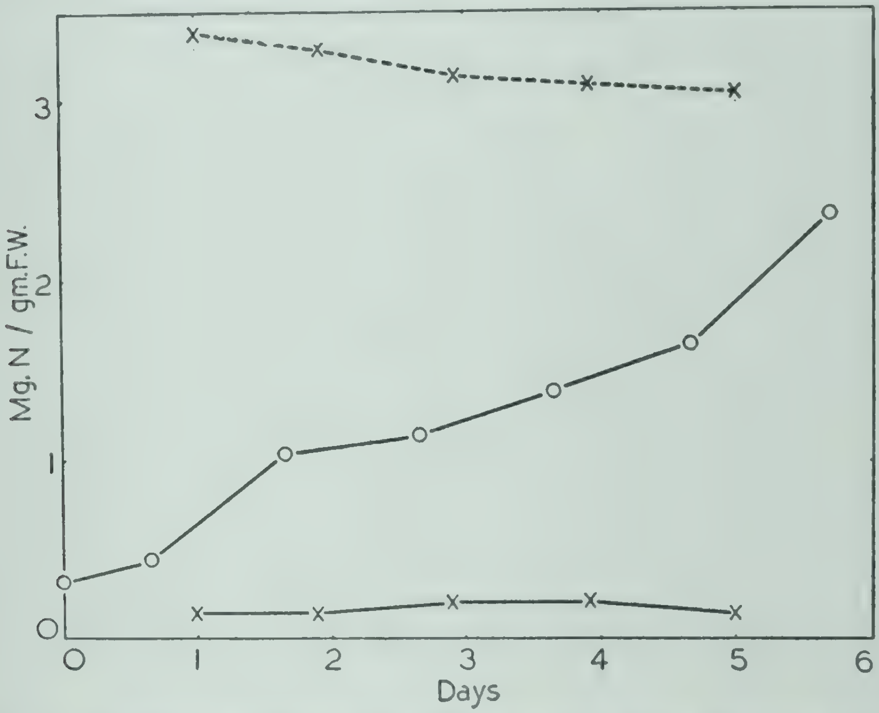

FIG. 17. Asparagine formation in cut leaves from barley seedlings about 4 weeks old. ○ asparagine-N in intact leaves; × in ground leaves under toluene. The slow rate of protein breakdown in the ground leaves, shown by the broken curve, may be compared with that of normal leaves in Fig. 15A. Data of McKee, 1937*a*.

Stability of the proteins seems to be the key to the length of the drift (see Table 15, p. 51). Unfortunately it is not at present possible to give any clear statement of the factors involved.[1] There seems to be fairly general agreement that in young leaves protein hydrolysis is slight, and that the tendency increases with age; conversely, the capacity for synthesis is high in young leaves and becomes less with age though it may persist in some degree, at least in detached leaves, so long as they remain green.[2] The difference between the drifts of leaves detached when young and when more mature seems to be closely related to their different degrees of protein stability.

It is not clear why this stability is reduced by cutting. Much has been written about the effect of carbohydrates in stabilizing and protecting the proteins of cut leaves; but recorded results cannot be said to bear this out. The time taken for the respiration of secondary substrates to become apparent is not lengthened by increased carbohydrate content. Wheat leaves with 1·3 per cent. total sugars showed no lengthening of phase 2 nor delay in arriving at phase 4 when compared with leaves having only 0·3 to 0·4 per cent. The change always happened after one day when the sugar-rich leaves still had some 0·8 per cent. of sugars as against 0·15 per cent. in the others. The sugar-rich leaves went on using both sugar and secondary substrates until their final collapse.[3] An extreme example is afforded by some barley leaves examined by Yemm. By a preparatory period of darkening while still attached to the plant, their available carbohydrates were reduced from 5·43 to 1·78 per cent. F.W. and their initial respiration rate from 0·95 mg. CO_2/gm. F.W./hr. to 0·54. This last value was below that of the normal inflexion point between phases 2 and 4, with the result that the CO_2-emission rate showed no fall, but a steady climb from the outset (Fig. 11, p. 46). The total difference of CO_2-emission between these and a similar batch of leaves kept in the light was 50·8 mg./gmF.W., and the difference of available carbohydrate was equivalent to 53·9 mg. From this it seems possible to infer that the breakdown of the secondary substrates was virtually identical at both levels of carbohydrate supply. Further, protein hydrolysis is at its fastest in barley leaves immediately after picking, when carbohydrates are abundant and are providing the whole of the respiratory carbon dioxide.[4] Mothes (1926) found that *Vicia faba* leaves, picked after active photosynthesis, also lost protein rapidly during the first 24 hours. Tobacco leaves, illuminated after detachment, showed massive increases of starch and sugars; but their losses of protein during the first 75 hours were as great as those of leaves kept in the dark.[5] Illuminated cherry-laurel leaves have been observed to yellow while still glutted with starch.[1] It is true that protein synthesis may have been induced in some cut leaves by supplying them with dilute sugar solutions.[2] This appears to occur more readily in young leaves than in mature or old, and is no indication that protein breakdown is not still occurring at the usual rate. There appears to be no convincing evidence that the presence of available carbohydrates retards protein hydrolysis, though it may sometimes, but not always, assist synthesis.[3]

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[1] There are discussions by McKee, 1937*b*, 1949; Chibnall, 1939; Petrie, 1939, 1943; Wood, 1945, who are able to come to no definite conclusions.

[2] Walkley, 1940, barley.

[3] Krotkov, 1939.

[4] Yemm, 1937.

[5] Vickery, Pucher, Wakeman, and Leavenworth, 1937.

The drift as hitherto described requires the presence of oxygen and depends entirely on aerobic respiration; yellowing does not occur in its absence. It is readily observed that any treatment, such as blocking the stomata of *Ficus elastica* leaves with vaseline, or immersion in water, which tends to reduce access of oxygen, retards yellowing. Michael observed that yellowing and proteolysis in *Tropaeolum* leaves was accelerated in 100 per cent. oxygen and retarded in 7 per cent. The yellowing of barley leaves may be entirely prevented by an atmosphere of nitrogen or by poisoning the oxidases with cyanide. The protoplasmic structure breaks down; but the dead and translucent leaves remain bright green and have a sweet, hay-like scent instead of giving off ammonia.[4] A similar result is observed with Sudan grass under nitrogen, and it has been shown analytically that the protoplasmic structure may finally break down without appreciable loss of protein or chlorophyll.[5]

The yellowing of phase 4 is associated with the accumulation of substances which, if formed at all in an attached leaf, would probably be largely translocated away. Among them the amides are notable and it has been found that their formation is suppressed by absence of oxygen,[5] the presence of chloroform[6] or grinding up the leaves;[7] all conditions which are known to delay yellowing. The association of yellowing with oxidative respiration suggests that it may be accelerated by a substance formed as an incidental of the aerobic metabolism. A water extract of yellowed cherry-laurel leaves has been found to hasten yellowing of freshly cut leaves allowed to take it up through the petioles.[8] Similar extracts of *Tropaeolum* also accelerated the yellowing of fresh leaves laid upon them; this effect was diminished by addition of glucose.[9] The same result was also produced by a 0·4 per cent. solution of asparagine and again was diminished by addition of glucose, apparently by removal of the asparagine, at least partly, in protein synthesis.

[1] Clapham, personal communication.

[2] Mothes, 1926 (young *Phaseolus multiflorus*); Paech, 1935 (small increases in several species); Pearsall and Billimoria, 1938 (*Narcissus*). But see also p. 246.

[3] Burström, 1943 (wheat).

[4] James and Hora, 1940.

[5] Wood, Cruickshank, and Kuchel, 1943.

[6] Mothes, 1925.

[7] McKee, 1950.

[8] Godwin, personal communication.

[9] Michael, 1935.

TABLE 17

Tropaeolum leaves floating on solutions of 0·4 per cent. asparagine and 1 to 3 per cent. glucose

Data of Michael

Treatment	*Duration (days)*	*Chlorophyll (mg./10 sq. cm.)*	*Protein-N*	*Yellowing*
Distilled water . .	3	0·06	0·26	. .
Glucose	3	0·17	0·41	retarded
Distilled water . .	4	0·50	0·79	. .
0·4 per cent. asparagine .	4	0·42	0·73	much accelerated
Analysed at start . .	0	0·46	0·83	. .
Glucose + asparagine .	2	0·45	0·89	retarded

There are thus reasonable grounds for supposing that the accelerated yellowing, characteristic of detached leaves, is autocatalysed by the production of amides. The volatile autocatalysts of fruit ripening are discussed on p. 75.

DETACHED ROOTS

Roots seem to have been rarely investigated in this way; but results have been obtained for maize by Girton.[1] Roots 2–4 cm. long, obtained from young seedlings raised aseptically, were transferred to respiration chambers and CO_2-drifts in tap water followed. The course was a simple one (Fig. 18), a rapid decline during the first few hours being followed by a steady long-continued and very gradual fall. Records were carried to 7 days and normally there was no secondary rise; if bacterial intrusions occurred they were indicated by a temporary hump.

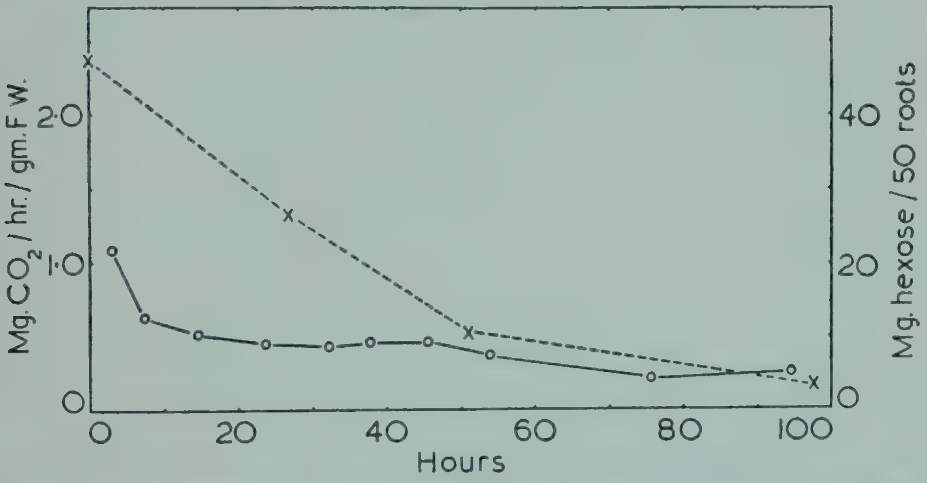

FIG. 18. CO_2-emission by maize roots, ○ ; and total sugar content as hexose equivalent, ×. After Girton.

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[1] Personal communication. I am greatly indebted to Prof. Girton for his permission to cite these unpublished results.

Available carbohydrates were in the form of sugars, mainly reducing sugars with a little sucrose, and showed a steady exhaustion that reduced the sugar concentration to about one-fifth during the first 2 days. In one experiment, the total CO_2-emission during this time was 54·8 mg. and the CO_2-equivalent of the simultaneous sugar loss was 52·8 mg. During a run of 172 hours the fresh weight of the samples was unaltered; but dry weight fell by 38 per cent. A very striking alteration was obtained when the roots were maintained in the presence of 2 per cent. sucrose+0·05 per cent. KNO_3. There were large increases of fresh weight, and dry weight went up by 59 per cent. in the tip region (0·5 cm.) and 52 per cent. in the young stumps, i.e. the next 2–3 cm. Such feeding also caused sustained increases of the respiration rate.

The general course of the drift curve resembles that of very young leaves (cf. Fig. 14) with its long sustained period of minimal CO_2-release. This is readily understood when the youth of the roots and their high percentage of meristematic tissue are remembered. A marked stability of the proteins appears to be indicated.

GERMINATING SEEDLINGS

The respiration rates of dormant seeds are very low, and during the early stages of germination there is always a rapid and prolonged rise. Expressed on a dry weight or similar basis this soon reaches the maximal value achieved throughout the life of the plant. The general course of events has been known a long time;[1] but not many species seem to have been examined in any detail, and, among those that have, the cereals are conspicuous. Apart from the technical importance of their germination in malting and agriculture, they present the experimental advantage that the active embryonic tissues are readily separated from their reserves.

An early study of the CO_2-drift during wheat germination was made by Rischavi (1876), who kept the germinating grains in the dark and followed the curve through to the point of exhaustion. The rate accelerated rapidly over the first 2–3 days and then more slowly until a maximum was reached after 10–11 days. After the peak a fairly rapid and continuous decline set in.[2] More detailed results are available for barley,[3] consisting of regular 3-hourly readings over 25 days. These have enabled the curve to be divided, like that for detached leaves (p. 42), into successive phases represented diagrammatically in Fig. 19.

Phase 1 lasts for about 2 days and consists of a rapid acceleration,

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[1] See, for example, Sachs, 1887, p. 399; de Saussure, 1833.

[2] For other results with wheat see Godlewski, 1882; Talmas (in Doyer, 1915); Bonnier and Mangin, 1884; Gindele, 1929; Met, 1950.

[3] W. O. James and A. L. James, 1940.

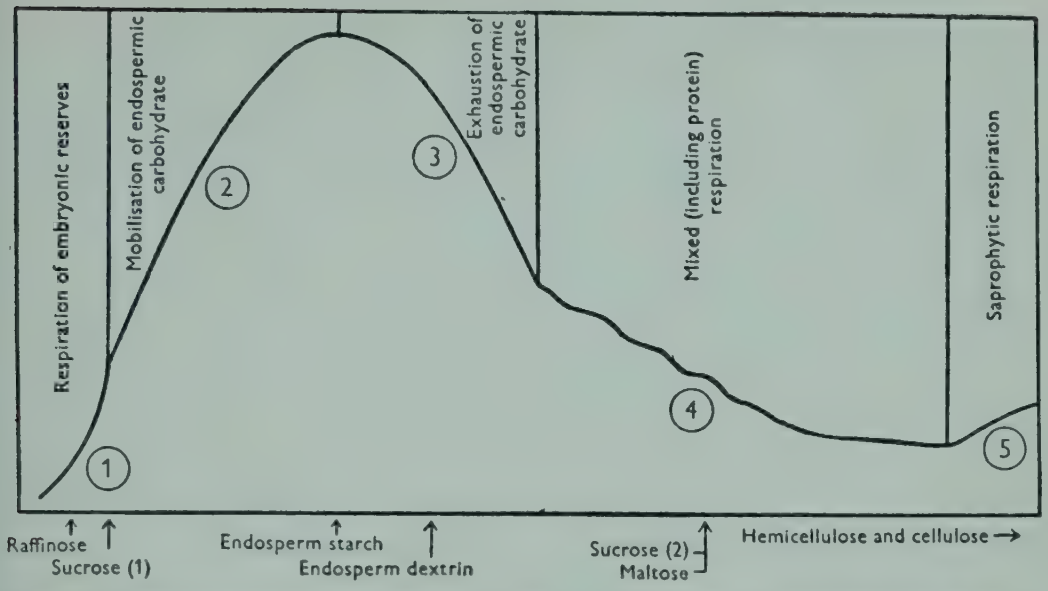
Respiration of embryonic reserves
Mobilisation of endospermic carbohydrate
Exhaustion of endospermic carbohydrate
Mixed (including protein) respiration
Saprophytic respiration
①
②
③
④
⑤
Raffinose
Sucrose (1)
Endosperm starch
Endosperm dextrin
Sucrose (2)
Maltose
Hemicellulose and cellulose →

FIG. 19. Diagrammatic representation of the CO_2-drift curve of barley seedlings germinating in the dark. The arrows show approximately the stages at which the substances named are exhausted.

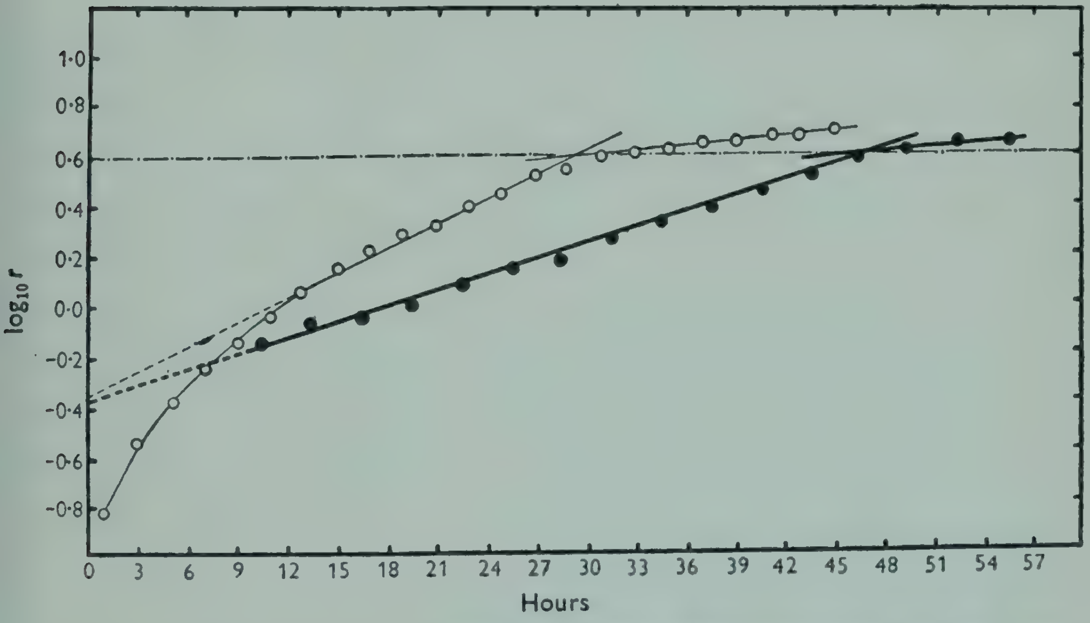

FIG. 20. Logarithm of the CO_2-emission rate of barley seedlings during the first phase of germination. The termination of the exponential rise depends on a critical rate of respiration and not on the time taken to reach it. ●–● grain of 1933; ○–○ grain of 1934. From James and James, 1940.

which from about 10–15 hours after wetting may be strictly exponential (Fig. 20). During the first 10 hours acceleration may be even faster. *Phase 2* is also a period of acceleration, easing to a peak after about

6 days. In some records, the transition from phase 1 to phase 2 is surprisingly abrupt, unlike those between the later phases. Phase 1 and phase 2 are distinguishable, though less well defined, in Rischavi's curve for wheat, and also in Barnell's records (1937) for barley. In *phase 3*, the rate of CO_2-emission falls off with increasing velocity until an interruption occurs. In our own results for Plumage Archer barley the peak value at the end of phase 2 is not maintained for more than a few hours and passes quickly into the decline of phase 3 (Fig. 21, curve A). In Rischavi's wheat curve (but not Met's) and in Barnell's and Forward's for barley (Fig. 21, curve B), the high values are kept up for longer periods. Curves of both types have been recorded for Spratt Archer barley by Folkes, Willis, and Yemm. In the short-peak curves, phase 3 may be more or less symmetrical with phase 2; but this seems to be fortuitous, and frequently the falling away is slower than the acceleration. *Phase 4* appears as an interruption of the decline, which here becomes less pronounced and is finally reversed in *phase 5* by a final rise.

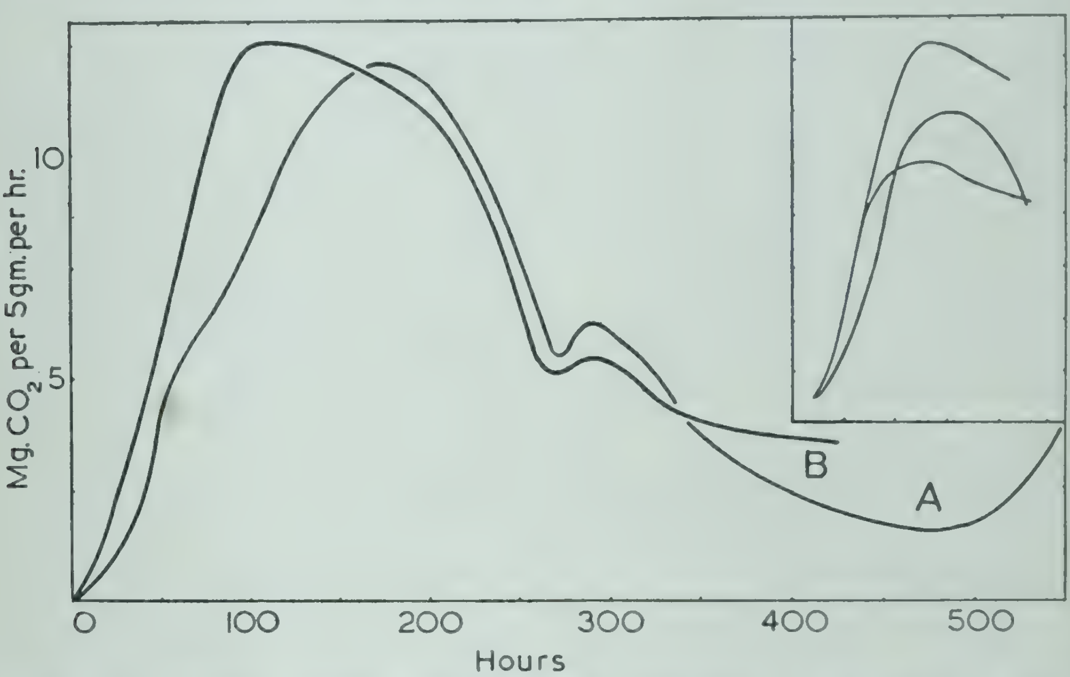

FIG. 21. CO_2-emission curves of barley seedlings germinating in the dark. A, after James and James, 1940; B, after Forward, 1951; inset after Folkes, Willis, and Yemm.

An interpretation of this drift may be arrived at by a consideration of analytical and other data and comparisons with the curve for detached leaves already described.

Phase 1. The first few hours are dominated by the wetting of the seed, and modifications of this phase can be brought about by alterations in the method of wetting. The results described represent what happens when total immersion of the grain is avoided and water is taken up from an aerated medium. Prolonged immersion may alter the picture considerably.

Frietinger (1927) found that wheat grains transferred to air after 15 hours in water might at first show an actual drop in the rate of CO_2-emission. Under the more normal conditions of our own experiments, it seems clear that the respiratory pace is set over the first 10 hours by the rate of water-uptake, determining the rate at which the embryonic cells pass from the dormant to the actively respiring state. Once the grain is saturated, the embryo is in a phase of unrestricted, i.e. exponential growth, and the respiration rate rises exponentially also. The free growth of the embryo at this time is indicated by the rapid increase of its cellulose and hemicellulose.[1] Exponential growth lasts as long as the reserves readily available to the embryo are in excess of the immediate requirement, i.e. for 2 to 3 days. During this time, and especially the earlier part of it, the reserves consumed are principally those present in the tissues of the embryo itself, which thus act as a sort of metabolic priming. They consist of a little fat, raffinose, and sucrose; but no starch. The starch-bearing cells of the barley endosperm show little or no spontaneous activity (Bruschi, 1908), and their evacuation depends mainly on the excretion of enzymes from the scutellum;[2] there is a pronounced lag before mobilization of their reserves becomes apparent.[3] During the first 24 hours the loss of reserves stored in the embryo is enough to account for about two-thirds of the new formation of polysaccharides and the CO_2 of respiration. During the second 24 hours

TABLE 18

Estimate of utilization of reserves in barley embryos.
Mg. per 100 embryos (hexose equivalent)

Data of A. L. James

First day				*Second day*			
Formed		*Broken down*		*Formed*		*Broken down*	
Cellulose	1·1	Raffinose	5·9	Cellulose	1·8	Raffinose	0·0
Hemicellulose	3·1	Sucrose	10·0	Hemicellulose	13·1	Sucrose	5·3
Maltose	4·4	Fats	2·8	Maltose	4·4	Fats	2·8
CO_2	20·7	Difference	10·6	CO_2	69·3	Difference	80·5
	29·3		29·3		88·6		88·6

One mg. fat assumed equivalent to 2 mg. hexose; embryo CO_2 assumed = 0·9 total CO_2 (cf. Barnell, 1937).

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[1] A. L. James, 1940.

[2] See Lehmann and Aichele, 1931, p. 514, for a summary of the evidence.

[3] Hora, 1936.

there is a considerable discrepancy which must be supplied from the endosperm, and thereafter the respirable material must be derived from the endosperm almost wholly.

Embryos isolated from their endosperms show only a low rate of CO_2-emission during the second day, which can be raised to about the normal level by feeding with 2–6 per cent. sucrose. When the supplies arriving from the endosperm are no longer adequately supplemented by those available *in situ*, growth and respiration are both restricted to the pace set by the rate of sucrose arrival.

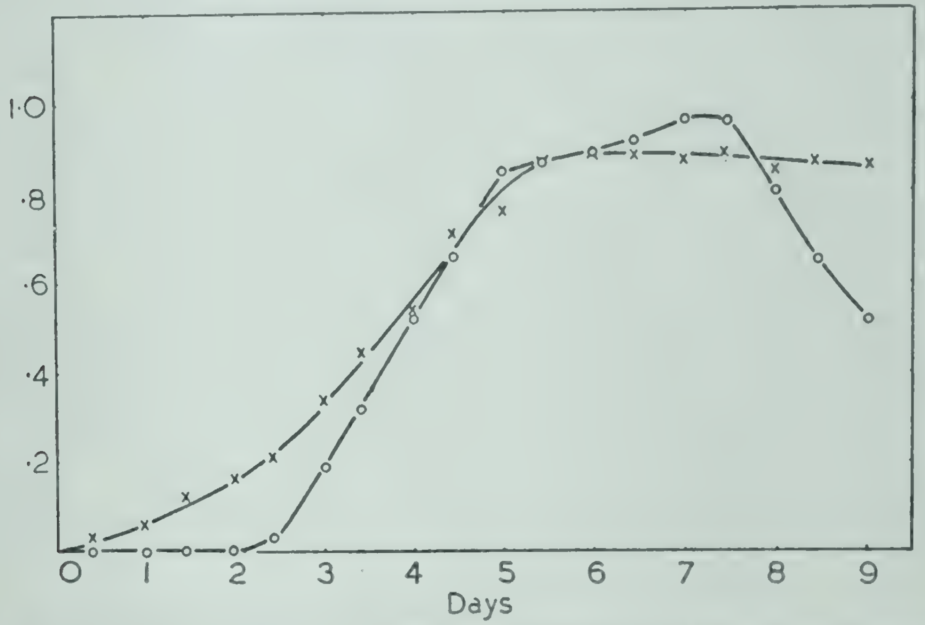

Fig. 22. Development of α-amylase (○) and invertase (×) in barley grains germinating at 21° C. Ordinates are relative initial rates of hydrolysis in the presence of excess starch and sucrose respectively. After Hora, 1936.

Phase 2 is the phase of mobilization of endospermic reserves. The respiration rate continues to rise, because the respiratory machinery is growing in the embryo and the mobilizing machinery in the endosperm. There is, for example, a rapid rise at this time in the activity of such hydrolysing enzymes as α-amylase and invertase in the endosperm (Fig. 22).[1] Starch is rapidly dissolved and soluble dextrins temporarily accumulate (Fig. 37A, p. 106). Acceleration of respiration in this phase is, however, no longer freely exponential, and is held in check by the rate at which carbohydrate from the endosperm is made available to the embryo. It is not yet certain whether it is the rate of hydrolysis or the rate of translocation that is limiting. It is not likely to be the rate of embryonic growth, since from about the third day the rate of

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[1] Hora, 1936.

CO_2-emission per mg. protein-N in the embryo rapidly falls off;[1] i.e. the ratio embryonic protein-N/CO_2-emission rises, and the amount of active protein cannot be limiting. The endosperm's proportion of the total CO_2-output throughout this phase is estimated by Barnell at no more than 10 per cent. The limit to the acceleration in the embryo will be set when carbohydrate supply can no longer increase to support the increasing rates of respiration and growth syntheses. This happens to coincide in our own experiments fairly closely with the time at which starch can no longer be detected, and dextrins reach their maximum concentration in the endosperm, and sucrose its maximum in the embryo. In Fig. 21 it is noticeable that the crest is attained earlier in curve B, with a slower fall, than in the abruptly peaked curve A; and this is confirmed by the results of Folkes, Willis, and Yemm (Fig. 21, inset). It therefore seems clear that when maximal or near maximal rates of respiration are maintained for some time, it is due to their being achieved at a relatively early time while there is still an abundance of endosperm reserves. It is not possible to say what factor ultimately controls these different accelerations of respiration; but it does not appear to be nitrogen content, since Folkes *et al.* found acceleration to be faster at high and low nitrogen contents and lower at medium.

Phase 3. The falling respiration rate of this phase corresponds with the disappearance of soluble reserves, particularly dextrins in the endosperm and sucrose in the embryo. The R.Q. remains slightly below 1. In these respects, phase 3 has obvious similarities with the first decline (phase 2) in isolated leaves. There are also significant differences. Although its respiration rate declines, the embryo continues to grow and synthesize cellulose and hemicellulose. A close similarity is also shown by wheat.[2] The embryo, although beginning to be starved, is still in a juvenile state, unlike most detached leaves.

Phase 4. Carbohydrate content of the endosperm is here reduced to a very low ebb, and the embryo cannot be replenished. Dextrins disappear from the former, and in the embryo itself sugars of all kinds are almost gone. Nevertheless, the fall in the respiration rate is to some extent arrested, and the R.Q. begins to fall, suggesting the utilization of non-carbohydrate reserves. The phase is thus analogous with the rising hump (phase 4) of isolated leaves. It is less pronounced than with mature leaves, and thus intermediate between the drifts shown by them and young ones. The fall of the R.Q. to 0·8 and analogy with other tissues make it probable that proteins provide most of the extra substrate at this stage. There also appears to be some breakdown of cellulose and hemicellulose. There is no secondary peak of respiration at this stage, and a declining rate is continuous until interrupted finally by collapse of the cytoplasm and the activity of intruding saprophytes (phase 5).

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[1] Folkes, Willis, and Yemm, 1952.

[2] Separate rates of embryos and endosperms are shown by Met, 1950.

Detached embryos

In their classical study of the barley grain, Brown and Morris (1890) showed that it was possible to germinate the embryos isolated from their natural source of nourishment in the seed.[1] They found that given no external nourishment other than water the embryos would germinate and survive for about 10 days; given carbohydrates, the best of which was sucrose, they would grow more and longer, and increase their dry weight.

During the development of the grain, the cells adjacent to the scutellum of the embryo are exhausted and crushed, forming at length a non-living layer having no organic connexion with the embryo. The relation of the germinating embryo to the endosperm is virtually that of a parasite extruding exo-enzymes into the host tissues, much as a spider treats a fly. As soon as the seed is partially moistened, it becomes easy to sever the two organs without damage to any living cells. Provided this is done aseptically the subsequent course of respiration may be followed in the usual way.

In the earliest stages (first 12 hours) respiration rate is dominated by the rate of water uptake as in complete grains. Both processes may be a little faster as a result of the removal of surrounding tissues.[2] A rapid respiratory rise then sets in which soon reaches its peak in embryos receiving only mineral nutrients; but which may be raised threefold by the presence of sucrose. As the rise is exponential the phase bears close similarity to phase 1 in the intact grains. It is, however, followed by an immediate fall (Fig. 23) which is easiest to observe in the higher-pitched sucrose curves; but which is also present in the embryos wholly dependent on their own reserves. This appears to last about 4 days, during which carbohydrate starvation in the unnourished embryos becomes acute, and includes breakdown of even hemicellulose and cellulose (Fig. 37B, p. 106). From the fifth to the eighth or ninth day, there is a secondary rise during which the R.Q. remains very low, and no doubt protein breakdown is setting in. This is followed by a long-continued final fall.

If the embryos are allowed to remain on their own endosperms till a later stage, the initial point of the curve is correspondingly raised. Fig. 24 after Barnell shows the result of detaching the embryos after about 2 days' germination, i.e. when they have completed phase 1. Since the respiration rate at this time depends on the rate of arrival of substrates from the endosperm, it shows no increase after detachment; but a typical starvation fall sets in at once lasting until the sixth day. The secondary rise and final fall then follow in due course, and are of about the same magnitude as in the embryo excised earlier and kept on

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[1] They did not claim to be the first to do this, and recorded that A. Gris had germinated isolated embryos of *Mirabilis jalapa* on moist sponge in 1864.

[2] R. Brown, 1943*a*.

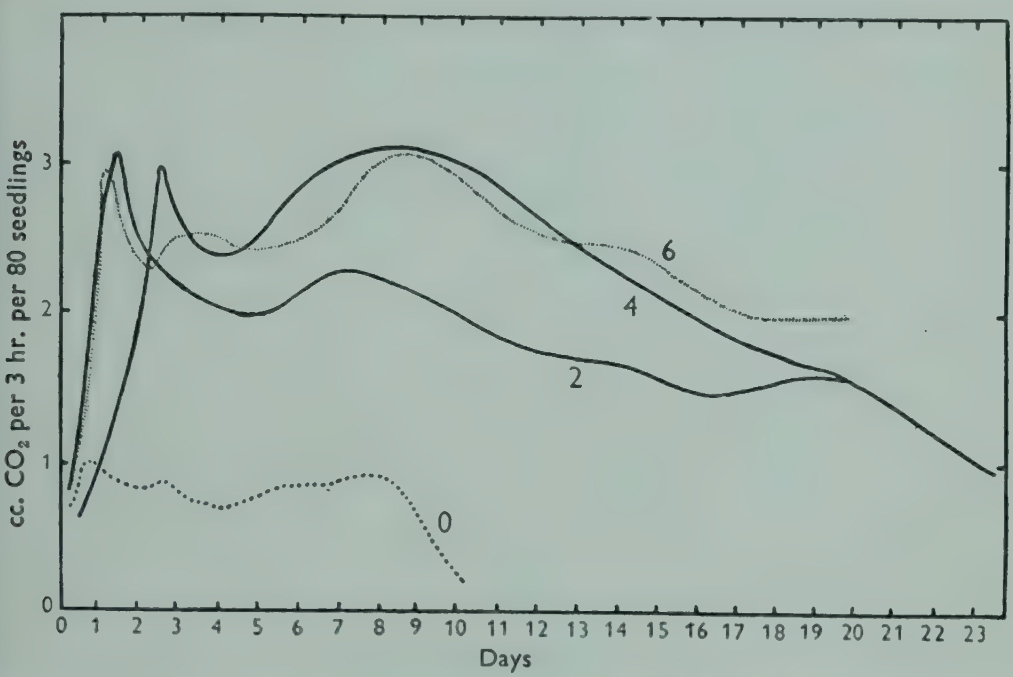

FIG. 23. Drift of CO_2-output by detached barley embryos receiving complete mineral culture solution and percentage additions of sucrose as shown. From James and James, 1940.

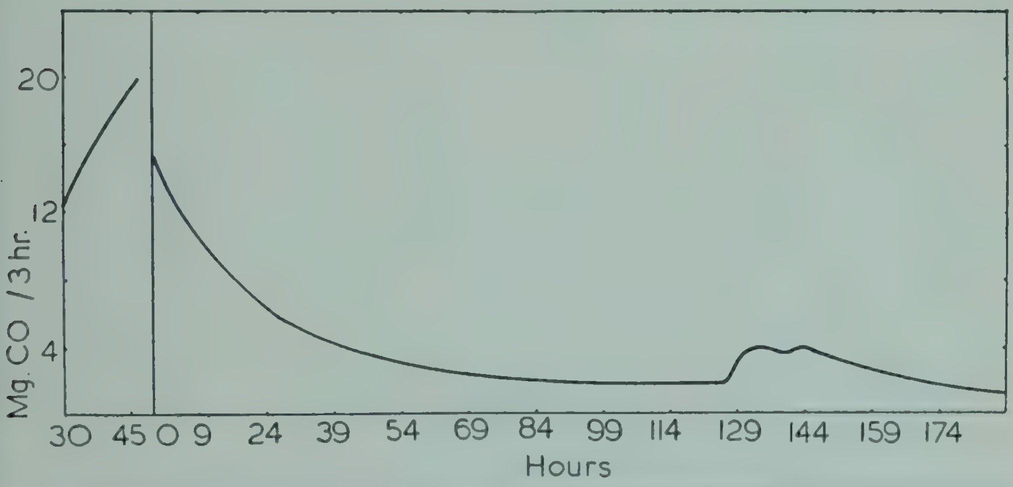

FIG. 24. Drift of CO_2-output by 100 barley embryos detached after 2 days' germination. Smoothed curve from the results of Barnell, 1937.

sucrose; both being much greater than the corresponding amplitudes with completely starved embryos. This results from the greater amount of synthesis achieved.

In comparing these drifts with those of intact grains, the absence of phase 2 is noticeable; even when sucrose, which Brown and Morris found to be the most successful carbohydrate, is given with complete minerals, a clear phase 2 does not show up. Growth also ceases unless the embryos are illuminated, although normally the embryos of intact grains continue to grow and increase their dry weight in the dark well into phase 3. The part played by hormones in this situation does not yet seem to have been cleared up.[1] A second difference of the drifts of isolated embryos from those of intact grains is the presence of a well-developed respiration hump instead of a mere delay in the starvation fall. The comparison is similar to that between detached cherry-laurel and detached barley leaves. It may similarly result from a greater lability of the endospermic as compared with the embryonic proteins.

FRUITS

Since it was realized in the nineteen-twenties that their respiratory drifts provided a rational basis for the storage of edible fruits, study of these drifts has been intensive. Apples have afforded the principal object in this country, and a number of tropical fruits, especially bananas, guavas, citrus fruits, avocados, and papaws, have been studied abroad. Tomatoes and pears have also yielded interesting results, and strawberries afford tissues of similar physiological behaviour.

The earlier stages of fruit development and respiratory drift show features similar to those of young seedlings; there is first of all a phase of rapid cell division (lasting in apples for 3–4 weeks after fertilization) during which the fruit increases slowly; this is followed by a phase of vacuolation and rapid enlargement; replaced in turn by a slow and prolonged maturation with relatively little change of size.

The similarities between these early stages and the corresponding stages of seedlings are obscured by the fact that most investigators of fruits have recorded their results in units of fresh weight, varying from a gramme to a kilo, and have not given the total respiration per fruit. Once the maximum weight is achieved at the end of the second phase and maturation sets in, this is of little moment. Many studies of fruit drifts only start during the maturation stage, i.e. after the fruit has been harvested in the ordinary way. Such drifts may profitably be compared with those of leaves detached after reaching a similar stage of maturity (pp. 40 et seq.). Clendenning's (1942) studies with tomatoes showed that detachment at such stages caused little alteration in the drift curve. Removal of immature fruit from the parent plant may, of course, result in profound changes in their drifts.[1]

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[1] See R. Brown, 1943*b*.

In fruits picked at normal harvesting times and maintained at moderate temperatures (10–25° C.), the drift usually follows such a course as is indicated in Fig. 25. There is a gradual fall followed by a dramatic rise, which in turn gives place to a second decline. Following the proposal of Kidd and West (1930), the rise following maturity is usually called the climacteric and the final fall senescence. The climacteric rise has been shown to occur in detached apples,[2] pears,[3] plums,[4] tomatoes,[5] guavas,[6] bananas,[7] papaws,[8] avocados,[9] and strawberries.[10] Lemons, and probably other citrus fruits, do not show a climacteric rise of CO_2-production when kept in air; but do so under concentrations of oxygen exceeding 33 per cent.[11] It has also been shown that the climacteric occurs in fruits that hang long enough on the tree. Kidd and West (1945) enclosed Rome Beauty apples in respiration chambers while still attached to the tree, and were able to demonstrate a climacteric rise. By the simpler method of measuring the respiration rate immediately after picking, the climacteric rise has been shown to occur in attached tomatoes[12] and McIntosh apples;[13] but, according to Biale (1946), avocados do not soften and show no climacteric so long as they hang on

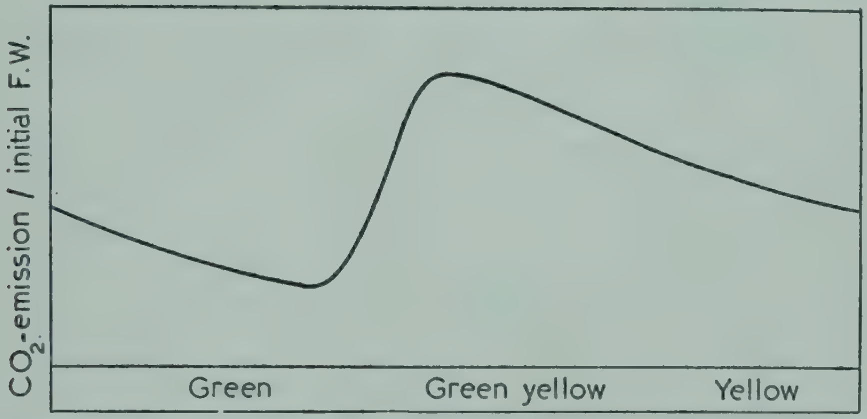

FIG. 25. Generalized diagram of CO_2-emission of apples picked at normal harvest in late summer and kept at moderate temperatures. The duration of the drift depends on storage temperature and on the variety of apple. Based on data of Blackman, Kidd, Krotkov, and others.

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[1] Cf. Krotkov, 1941 (apples); Kidd and West, 1935 (pears).

[2] Blackman and Parija, 1928; Kidd and West, 1930; Trout, Tindale, and Huelin, 1940.

[3] Kidd and West, 1935; Kidd, West, Griffiths, and Potter, 1940.

[4] Roux, 1940.

[5] Clendenning, 1941.

[6] Ranjan and Khan, 1934.

[7] Hartshorn, 1931; Gane, 1936; Wardlaw and Leonard, 1940.

[8] Wardlaw and Leonard, 1936.

[9] Biale, 1946.

[10] Overholser, Hardy, and Locklin, 1931; W. H. Smith, 1936*b*.

[11] Biale, 1950.

[12] Gustafson, 1929; Clendenning, 1942.

[13] Krotkov, 1941.

the tree. In pears, the climacteric coincides with the ideal degree of softness and aroma for eating, in apples and bananas the most desirable condition immediately follows the peak. The general similarity of these changes to phases 4 and 5 of detached leaves (p. 40) is evident. The colour changes are similar also, the peak of the rise in apples corresponding with a 'green-yellow' tint. In making the comparison, it must be remembered that only the surface cells are coloured, whereas all the colourless cells of the flesh are contributing to the respiration.

Having in mind the drift curves of cherry-laurel leaves, Blackman and Parija (1928) interpreted the apple drift-curves as being the resultant of two opposing tendencies; firstly a progressive reduction of resistance to metabolic change and secondly a starvation factor. The first permitted the climacteric acceleration and the second imposed the senescent fall. The nature of the starvation was not apparent; and it was at once made clear that it could not be lack of sugars. In the following year Gustafson (1929) published results showing that the sugar content of tomatoes increased after the climacteric, and quoted earlier results of Sando to the same effect. Kidd and West (1930) showed that apples at the time of death had consumed only 16 to 20 per cent. of their available carbohydrate reserves; and, further, that apples stored at 2·5° C. had consumed five times as much when they reached the peak from which the fall begins as those stored at 22·5° C. This second point was important, because it had been suggested that it was only at the higher temperature that starvation was apparent. The sucrose concentration in bananas also goes on rising after the climacteric.[1]

In general, fruits with high respiration rates tend to have shorter storage lives than those whose drifts are pitched lower.[2] This is also true of different varieties of the same species. Kidd and West (1936) found that the respiration of Williams pears was faster and the storage life shorter than those of Comice and Conference. Coming to a single variety, the storage life and the rate of respiration are both functions of temperature. Kidd and West (1930) investigated Bramley's Seedling apples at three temperatures and obtained the following:

TABLE 19

Respiration and storage life of apples

Data of Kidd and West

Temperature	2·5	10	22·5° C.
Relative rate of CO_2-emission:			
Initial	1	2·1	5·6
At peak	1	1·8	5·4
1/Duration of storage life. . . .	1	2·1	5·1
Total CO_2-production	12·84	11·35	11·42 litres N.T.P.

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[1] Gane, 1936.

[2] See Biale, 1950, for summary.

The inverse relationship between respiration rate and duration of storage life is very beautifully demonstrated. It follows that approximately equal amounts of CO_2 will have been liberated at the time when the tissues collapse, regardless of the temperatures at which they have been maintained.

Two interpretations of this result seem possible; either the tissues collapse when some component has been reduced to a critical level, or, alternatively, the respiratory machinery wears out proportionately to the amount of change produced. It is not clear why the reduction of the respirable materials by some 20 per cent. should cause a breakdown of the tissues, and it has already been shown that the maintenance of the carbohydrate level in isolated leaves will not stave off senescence (p. 59). The idea that fleshy fruits in fact perform their entire drift with an excess of carbohydrates is supported by direct analysis, and by the observed R.Q.s. Mature apples[1] and strawberries[2] appear to maintain a R.Q. close to or slightly above unity. Green tomatoes have low R.Q.s due to acid formation; but these rise as the colour changes to yellow and thereafter remain very close to one. The rise is accelerated by raising the temperature to 30–33° C.; but does not go appreciably above one even at full ripeness.[3] There is no evidence of a steady drop at this stage similar to the fall to about 0·8 in detached leaves. It has been shown[4] that in pears the rise of respiration rate during the climacteric is out of all proportion to the small increases in sucrose and protein[5] that occur at the same time. The small increase of protein also affords an interesting contrast with the extensive proteolysis in yellowing leaves. It is in fact impossible to relate the drift to the internal concentration of any internal substrate yet examined. Attempts have been made to attribute it to alterations in the internal gas composition, which may also be reflected in external concentrations when the fruits are enclosed in respiration chambers. It has been suggested that, as the fruit enlarges and the surface/volume ratio gets smaller, there will be an inevitable fall of the internal oxygen concentration. This fall Wardlaw and Leonard (1936) regarded as a causal factor explaining the 'running down' of the system. They showed that the respiration and transpiration rates of papaws per gm. F.W. of tissue ran parallel with surface/volume. It is not clear that they took any precautions when comparing fruits of different sizes to have them at the same developmental stage. It is likely that the smaller fruits were also the younger ones, and that their higher respiration rates illustrated an age drift rather than a correlation with specific surface. Gustafson (1926) could not find any definite relationship between size and respiration rate in tomatoes. The flesh of most fruits is well aerated by intercellular spaces, so that resistance to gaseous invasion is virtually limited to the skin; and the high Q_{10}'s of fruit respiration (Table 3, p. 26) show that inward diffusion of oxygen can rarely be a restricting factor.

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[1] Hackney, 1944*a*; Watson, 1932.

[2] Gerhart, 1930; Overholser, Hardy, and Locklin, 1931.

[3] Gustafson, 1936.

[4] Kidd, West, Griffiths, and Potter, 1940.

[5] See also Hulme, 1936; Kidd, West, and Hulme, 1938; J. F. Turner, 1949; Robertson and Turner, 1951, for apples.

The skin of apples is usually side-tracked by an opening at the calyx communicating with the core. It has been shown[1] that Delicious apples, which have open calices and also open lenticels in the skin, have fast respiration rates, which show no correlation with internal oxygen concentrations. Granny Smith apples provided a contrast, having closed calices and lenticels that were usually closed also. During their maturation there was an observable correlation between respiration rate and internal oxygen concentration, both of which were low. The oxygen concentration was further reduced by coating the surface of the fruit with shellac and blocking the calyx. Respiration rate then slowly declined, though much more slowly than oxygen concentration. The result of these manœuvres was actually to delay ripening, and this was in accordance with much previous experience on the effect of changing oxygen concentrations.[2] We cannot, therefore, attribute the development of the climacteric to internal shortage of oxygen.

The suggestion of Kidd and West (1930) that the climacteric rise and the fall of respiration rate leading to the final collapse are both due to progressive deterioration of protoplasmic condition is more defensible. This has much in common with Blackman's[3] notion of a reduction of organization resistance. He defined his resistance as an impediment to metabolic rate, adding, 'We may picture some of this hindrance to reaction as achieved by impermeable protoplasmic membranes. More significant, however, will be the adsorption or combination of one or both of the reactants by the stabilised components of the protoplasm.' Kidd and West spoke similarly of the deterioration as 'principally due to changes in colloidal state of the protoplasm'. In arriving at this conclusion they laid particular stress on the lack of correlation between the drift and variation of any discoverable substrate concentration, and also on the very high temperature coefficients of the acceleration of respiration in approaching the peak. The Q_{10} of initial respiration rates were as usual a little over 2; but the Q_{10}'s of the climacteric acceleration at the two temperature intervals, 2·5–10° C. and 10–22·5° C., were of the orders 10 and 100 respectively. Such values could only be attributed to a dependence on colloidal change.

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[1] Hackney, 1943*a* and *b*, 1944*a* and *b*.

[2] Kidd and West, 1933, 1945 (apples); ibid. 1936 (pears); Clendenning, 1941 (tomatoes); also Biale and Young, 1947 (lemons).

[3] Blackman and Parija, 1928; see also discussion, p. 13 *ante*.

The initiation of these changes might be due to the removal of a stabilizer which was used up at a rate more or less proportional to that of respiration. The suggestion has been made[1] and maleic acid named as a candidate for the position[2] because it was found to retard the yellowing of apples and bananas. Much greater support has been found for the alternative proposal that the protoplasmic changes are induced by the progressive formation of an accelerator.

Ethylene formation

Fruits give off volatile and aromatic substances in quantities that increase as ripening proceeds, and that reach a maximum at the peak of the climacteric. Many of these substances remain unidentified; but the presence of ethylene among them has been proved beyond doubt. The interest of the observation lies in the fact that ethylene has also been shown to catalyse CO_2-production and accelerate the onset of yellowing and the climacteric.

It is a well-known fact, easily verified, that a few ripe fruits enclosed among green ones will hasten their ripening. Cousins (1910) was perhaps the first to put the observation on record. Artificial yellowing of lemons was practised in the first decade of the century by subjecting them to the vapours of paraffin stoves, and it was shown that the products of incomplete combustion (not the heat) were responsible;[3] among them was ethylene which was proved effective in extreme dilutions. A concentration of 2×10^{-7} caused the yellowing of lemons in 14 days (Denny, 1924). Acceleration of ripening by traces of ethylene has also been demonstrated with oranges,[4] apples,[5] pears,[6] bananas,[7] and Japanese Persimmons (*Diospyros kaki*).[8] To be effective, applications must be made at an early stage of maturity when they accelerate respiration rates; applications of ethylene after the climacteric peak has been passed no longer accelerate respiration.[9]

The identification of ethylene among the volatiles given off has been accomplished with special thoroughness by Gane (1934, 1935) using Worcester Pearmain apples. Concentrations around 10^{-6} in air have well-marked biological effects which aid identification. They inhibit epinasty in *Mimosa pudica*, *Ricinus communis*, and tomato plants, and have well-marked effects on the growth of many seedlings. These effects were all observed on passing the volatiles from apples over test plants. Conversely, ivy plants and seedlings of barley, maize, and other cereals, which are unaffected by 1 part per million of ethylene, showed no response to apple vapours. Numerous other known components, including acetaldehyde, alcohols, and esters, had no effect on the ethylene-sensitive plants.

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[1] Roux, 1940. [2] Copisarow, 1936. [3] Sievers and True, 1912.

[4] Miller, Winston, and Schomer, 1940.

[5] Kidd and West, 1933, 1934, 1937.

[6] Kidd and West, 1938; Hansen and Hartman, 1937.

[7] Gane, 1937; Regeimbal, Vacha, and Harvey, 1927.

[8] Davis and Church, 1931. [9] Hansen and Hartman, 1937.

The active substance was not destroyed by water; but was removed by bromine, ozone, and fuming nitric and sulphuric acids. Complete chemical identification was obtained by passing the air-stream leaving ripening apples for 4 weeks into bromine maintained at −65° C. This procedure yielded an oil, ethylene dibromide, which on heating with aniline gave a solid crystallizing from dilute alcohol. The m.p. = 62·5° C. was unaltered on fusing with NN′ diphenylethylenediamine. The product also yielded an oxalate agreeing with a known preparation. Satisfactory chemical identification of ethylene has also been obtained with bananas[1] and avocados.[2] Hansen and Christiansen (1939) recorded rates of ethylene production for apples and pears varying from 0·001 to 0·28 ml./gm./hr.[3]

The following considerations connect the climacteric with internal production of ethylene. The latter begins at a very early stage, and has been detected in 30 gm. apples still showing active cell division.[4] The rate of formation increases as the fruit ages, especially during the period of the climacteric.[5] There is no simple stoichiometric relation between the amounts of ethylene and carbon dioxide produced; but the two rates of release follow more or less parallel courses up to the peak of the climacteric. Thereafter CO_2-output diminishes, but ethylene output may be maintained. Ethylene formation thus appears to be autocatalytic so long as respiration can be stimulated. Further points are that early-ripening fruits produce more ethylene than slow-maturing ones;[6] a rise of temperature up to about 20° C. sharply accelerates both ethylene production[7] (Q_{10} about 5) and onset of the climacteric (Q_{10} about 10),[8] while conversely the absence of oxygen greatly retards both.[9] Finally, ethylene produced by any fruit will accelerate the ripening of any other of the same or different species;[10] and any treatment like bad aeration that tends to retard the escape of ethylene hastens spontaneous ripening also.

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[1] Niederl, Brenner, and Keeley, 1938.

[2] Pratt, Young, and Biale, 1948.

[3] See also Biale, 1950.

[4] Kidd and West, 1934, 1938, 1945; Gane, 1938 (pears).

[5] Hansen and Hartman, 1937 (pears); Hansen and Christiansen, 1939; Nelson, 1940; Hansen, 1945 (apples).

[6] Nelson, 1939; Hansen, 1945 (apples); Hansen, 1942 (pears).

[7] Hansen, 1945.

[8] Kidd and West, 1930.

[9] Hansen, 1942 (pears).

[10] Gane, 1937, 1938.

The mechanism of the ethylene effect is not yet fully explained. It is not associated with any definite changes in the concentrations of sugars or other possible substrates,[1] and it has not been shown to depend on the activation of any specific enzyme; though results are conflicting.[2]

Since the climacteric is believed to result from a change in the colloidal state of the protoplasm, it is interesting to inquire whether ethylene can produce such changes. An increased cell permeability due to ethylene has been shown by Nord and Francke (1928). An observation by Huelin and Barker (1939) is also significant. They found that potatoes with a high sugar content, induced by precooling to +1° C., showed no increased CO_2-output on treatment with one part of ethylene per thousand. As the sugar content slowly dropped to the low level normal for 15° C., the ethylene effect again became evident, being first detectable with about 2 per cent. internal sugar. This confirmed an earlier experiment by Herklots (1928), and suggests strongly that the action of the ethylene is upon whatever controls the supply of substrate to the respiratory enzymes, rather than upon the substrates or enzymes themselves. This could also explain why the effects of short or mild doses are reversible, but those of repeated or prolonged doses irreversible.[3] A secondary effect of ethylene, the softening of the tissues, is explained by the conversion of insoluble to soluble pectin.[4]

YOUNG AND ADULT SHOOTS

The classic investigation of these stages is that of Kidd, West, and Briggs upon *Helianthus annuus*, recorded on the basis of the respiratory index (p. 19). The authors refer to their material as the 'plant as a whole'; but cut it above ground-level. The index was highest from the second to twenty-second day after germination and thereafter fell away rapidly (Fig. 26) until it was about 13 per cent. of its initial value. The fall was continuous, and there were no secondary rises. When the plants had attained a fair size, stems and leaves were taken separately. Both showed similar descending series; but the stems always had a lower respiratory index than the leaves, due, at least in part, to their higher proportion of non-respiring tissue (wood and fibre). This kind of dilution is not the only cause of decline. The stem apex (top cluster of leaves) also showed a steadily declining respiration, though it remained mainly meristematic, and did not show progressive differentiation like the stem taken as a whole and the leaves. Similar declines of respiratory indices in meristems were observed by Singh (1925) in twelve other species.

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[1] See, e.g. Dustman, 1934; Regeimbal and Harvey, 1927.

[2] Englis and Zannis, 1930; but cf. Iwanoff, 1932.

[3] Kidd and West, 1937; Huelin and Barker, 1939.

[4] Hansen, 1939*a* and *b*.

The progressive decline in the apex is necessarily reflected in the respiration of the series of organs produced from it. This no doubt applies to stem segments; but is most easily demonstrated in leaves. Each successive leaf rudiment must start at a lower respiratory level than the one before; and this may still be evident in the expanded leaves. Richards (1938) examined successive leaves during the growth of the barley plant at a stage judged from external appearance to be the same for each, viz. 'the time of complete emergence'. The CO_2-output (mg. CO_2/gm. D.W./hr.) showed a steady decline as the series progressed, which was repeated by a series of leaves examined at a slightly later stage, i.e. when they were placed one node lower down. We therefore have a pattern of respiratory drifts more or less parallel with that of the apex. The character of the drift is not always so simple;[1] but, whatever its exact form, it is evident that a set of leaves taken down a stem cannot be regarded as giving stages of a simple time series, since each begins with a different respiration rate. Such a set gives a picture of the distribution of respiration rates in the plant

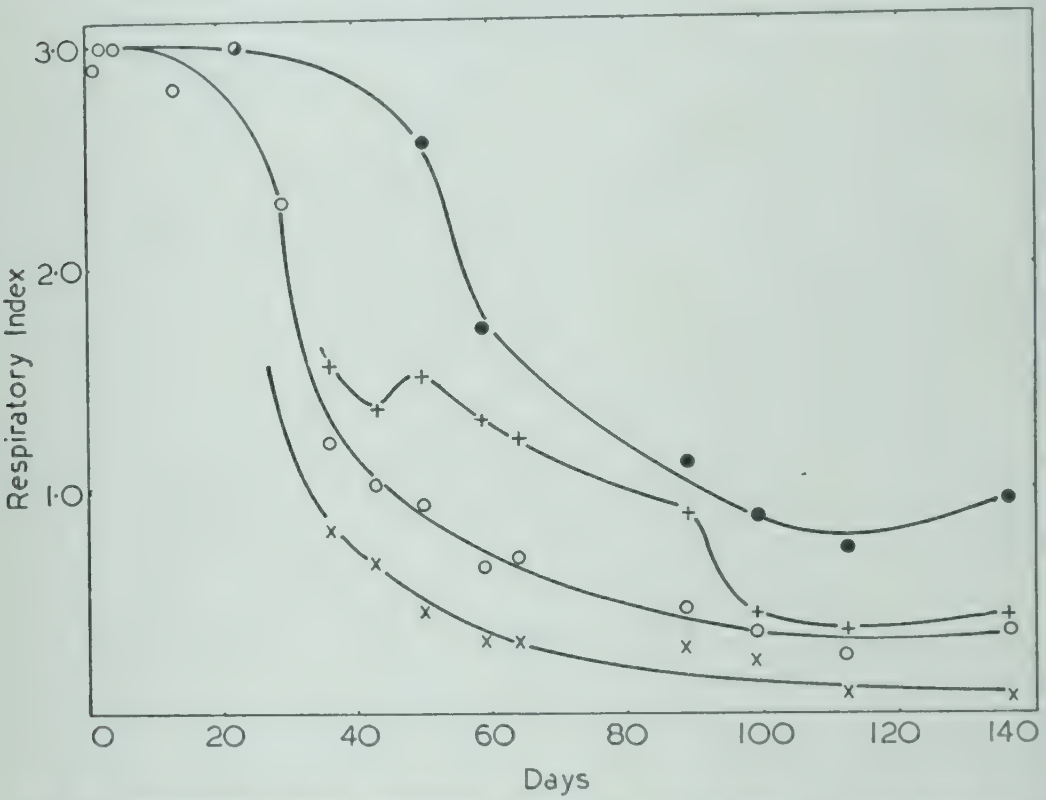

FIG. 26. Respiratory index of sunflower plants. ○, whole shoot; ●, stem apex; +, leaves; ×, stem. Data of Kidd, West, and Briggs, 1921.

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[1] Cf. Gregory and Sen, 1937, also for barley.

at any one time; but cannot be used to infer the nature of the drift in an individual leaf. Fig. 27 shows the distribution in sunflower and sorghum plants of medium age and brings out the interesting point that, whereas sunflower shows a steady decline from apex to base, sorghum appears to have a minimum in the lower half of the range with a moderate rise lower down. A similar secondary maximum was observed in the lower leaves of maize; but the corresponding stem segments showed a steady decline to the base. These relatively high rates in the basal leaves might be due to a secondary rise similar to that of phase 4 in detached leaves.

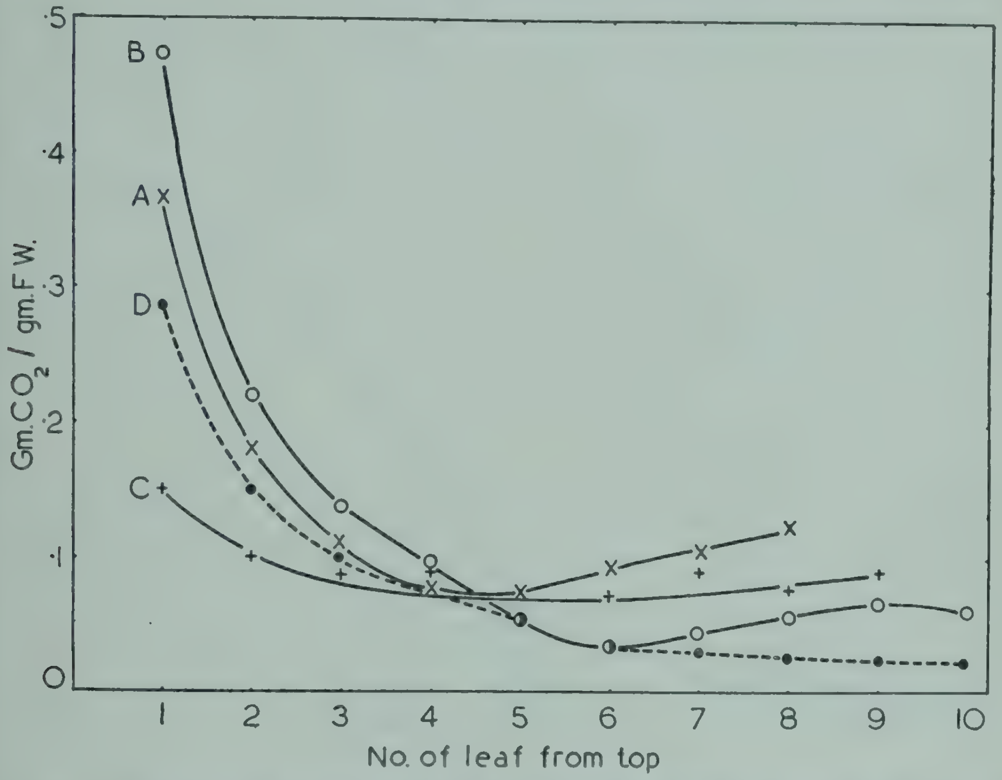

FIG. 27. CO_2-production of leaves for first 24 hours after removal from the plant. Curve A. ×, leaves taken from 4 young sorghum plants, 1 ft. high with 8 leaves; B. from sorghum plant 3 ft. high with 10 leaves; C. from plant developing tassels, with 9 leaves; D. from half-grown sunflower plant with 10 leaves. Results of Hover and Gustafson.

Complete CO_2-drift curves for leaves still attached to the plant have been determined by Arney (1947) with strawberries. The life period was about 120 days, and the whole curve is similar to that for isolated and darkened leaves; but very much longer drawn out. There is a period of rapid respiration while the young leaf is unfolding and expanding. Respiration rates were expressed as c.c. CO_2/sq. dcm./hr.; but, even so, rapidly rising rates were recorded in the early stages of leaf expansion, when cell number had probably already reached its maximum and vacuolation was the dominant activity. The rise of respiration rate ceased at about the same time as leaf expansion, and thereafter the rate fell away fairly rapidly to a level about half the maximal. This slowly declined till about the eightieth day, when a secondary rise set in simultaneously with yellowing. Leaves expanding in May to July on older plants dried and withered at the end of their cycle without yellowing and showed no secondary rise of respiration rate (see Fig. 28 for sample curves). The similarities with the accelerated behaviour of cut leaves is evident.

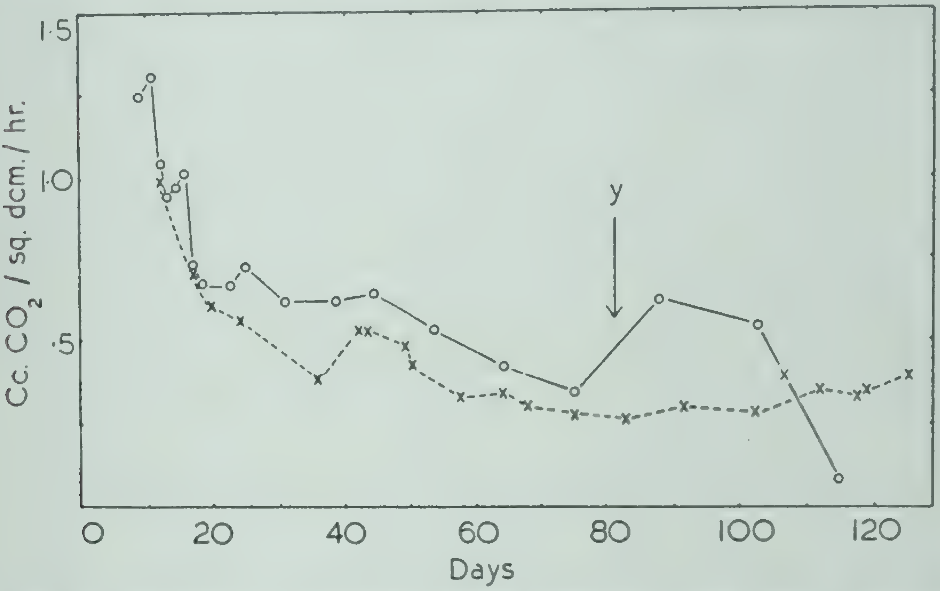

Fig. 28. CO_2-production of strawberry leaves attached to the plant. Two curves selected from numerous records given. ○, leaf formed on young plant in September, lasting until following January; ×, leaf formed on old plant in July, dying without yellowing. The point at which September leaves yellowed is indicated by *y*. After Arney, 1924.

Comparing the various drifts shows that three internal factors dominate the rate of respiration: the concentration of a substrate, increase of catalytic protoplasm, and change of protoplasmic condition. The influence of substrate concentration is particularly evident in the starvation of detached leaves and seedlings kept long in the dark; first in the decline due to carbohydrate loss and later in the second decline due to loss of nitrogen compounds. Increase of protoplasm is a dominant factor during the germination of seedlings, the first stages of fruit formation, and the unfolding of leaves. It results in a rising respiration rate during the earliest parts of their drifts. Secondary rises have to be attributed to what we can at present only vaguely call change of protoplasmic condition. The effect of protoplasmic structure on metabolic rates in general is discussed on p. 13. A slow drift of changes always goes forward in maturing protoplasm and appears to be catalysed in some tissues by the formation of accelerators as by-products of aerobic metabolism. In fleshy fruits ethylene fulfils this role (p. 75). It will also accelerate the yellowing of leaves,[1] but is not known to be formed by them. Detached leaves accumulate non-volatiles which have a similar effect, and which may be acid-amides (p. 60). Detached leaves and stored fruits usually run through their drifts more rapidly than if left on the parent plant. This is attributable, at least in part, to the faster accumulation of the accelerators after detachment and enclosure. In detached leaves the accumulation of the accelerator runs concurrently with starvation, so that the respiratory rise is immediately followed or even interrupted by a final and drastic fall. In fruits the accelerator is formed in sufficient quantities to produce the secondary rise at an early stage before the substrates are seriously depleted, and the decline following the climacteric may be long drawn out. Leaves show a chemical breakdown of protein leading up to the secondary rise; but in fruits there is an actual synthesis of protein during the climacteric (p. 73), and the Q_{10} of its acceleration is too high to depend on a chemical reaction velocity. While, therefore, there are similarities between the respiratory hump of leaves and the climacteric of fruits, and both probably originate in a self-induced change of physical state, it is equally probable that the type of substrate drawn upon differs in the two organs.

After the respiratory peak is passed, deterioration of the protoplasm goes on until it is terminated in both leaves and fruits by a final collapse frequently made evident by the rapid CO_2-production of saprophytes flourishing upon the remains.

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[1] Herklots, 1939 (cherry-laurel, *Sequoia gigantea*).

IV

THE RESPIRATORY QUOTIENT

THE respiratory quotient is the volume, or molecular, ratio of carbon dioxide produced to oxygen simultaneously consumed. It can be determined with relative ease and accuracy. Early workers used simple eudiometers, which were later replaced by accurate gas analysis by Haldane's, and similar, constant-pressure techniques; more recently still, constant-volume manometry has become popular. The R.Q. has the great advantage of being a pure ratio independent of the amount of respiring material. Comparisons may therefore be made between very different tissues without any difficulties about units such as afflict comparisons of rates.

Opinions of the value of R.Q. determinations vary. When studying the effects of oxygen shortage on the respiration of apples, Hackney (1943) remarked: 'It must be remembered that alcohol analyses are not necessarily a true index of anaerobic respiration, and that only determinations of the respiratory quotient can be regarded as a true index of the nature of respiration.' This is placing it high; at the other extreme is Barnes's (1905) view. 'This respiratory ratio has proved a veritable will-o'-the-wisp, leading investigators into a bog where their labours and their thinking were alike futile.' It is well, therefore, to be on guard.

One of the main dangers arises from the fact that the quotient may be affected not only by chemical but also by physical causes, particularly by the different solubility and diffusion coefficients of the two gases. These last are usually most important when respiration rates are rapidly changing, as during germination or the transfer of a bulky tissue from one temperature to another. When respiration has settled to a steady rate, the R.Q. is usually determined by the chemical nature of the substrates being consumed. At low oxygen pressures the results are complex, because anaerobic processes release additional carbon dioxide; but above 5–10 per cent. oxygen, complete oxidation of the respiratory substrate is likely to dominate. Occasionally, anaerobiosis may occur even in air, and many meristematic tissues have been shown to have high R.Q.s accompanied by an accumulation of alcohol.[1]

A general survey of R.Q.s leads to a result of considerable importance. In air and at moderate temperatures, many adult tissues show an R.Q. approximating closely to 1·0. Exact unity of the ratio agrees with the

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[1] Ruhland and Ramshorn, 1938.

TABLE 20

Examples of respiratory quotients about 1·0

		CO_2/O_2	
ADULT LEAVES .	*Pelargonium zonale*	1·05	Ullrich and Ruhland, 1928
	Tradescantia viridis	1·00	,, ,,
	Wheat	1·00–1·173	Moyse, 1950
	Vicia faba	1·02	Yemm, 1934
	Buxus sempervirens	1·03	A. L. James, 1937
	26 species	1·00–1·11	Maquenne and Demoussy, 1913
	Polygonum fagopyrum	1·101	Moyse, 1950
	Tomato	0·94–1·03	Caldwell and Meiklejohn, 1937*a*
STEM . .	Pea, etiolated internodes	0·98–1·07	Christiansen and Thimann, 1950
RHIZOMES .	*Convallaria maialis*	1·00	Bonnier and Mangin, 1884
	Polygonatum vulgare	0·98–1·00	,, ,,
TUBERS .	Potato	1·00	Barker, 1936
ROOT . .	Carrot	1·01	Turner, 1940
BULB . .	*Narcissus*	0·96	Stich, 1891
SPADIX .	*Sauromatum*	1·0	van Herk, 1937
	Arum maculatum	1·09	James and Beevers, 1948
FLOWERS .	*Hyacinthus orientalis*	1·00	Bonnier and Mangin, 1884
	Robinia pseudacacia	1·00	,, ,,
MATURING FRUITS .	Apple	1·00	Hackney, 1944*a*
	Banana	1·04	Langworthy and Milner, 1912
	Tomato (at 30–33° C.)	1·03–1·06	Gustafson, 1936
FLESHY RECEPTACLE	Strawberries	0·909	Overholser, Hardy, and Locklin, 1931
ALGAE Green .	*Scenedesmus quadricauda*	0·94–1·09	Taylor, 1950
	Spirogyra (vegetative)	1·00	Pal, 1934
	Enteromorpha linza [1]	0·943–1·097	Hoffmann, 1929
Brown .	*Fucus vesiculosus*	0·991–0·928	Harder, 1915
	,, ,, (fruiting)	1·021	Hoffmann, 1929
	Fucus serratus [1]	1·035	,, ,,
Red . .	*Gigartina tiedii*	0·975	Kniep, 1914
	Polysiphonia sp.	0·998	Harder, 1915
	Polysiphonia violacaea	1·022	Hoffmann, 1929
	Ceramium rubrum [1]	1·023	,, ,,
FUNGI . .	*Phycomyces blakesleeanus* (on bread)	0·99–1·21	de Boer, 1928
	Aspergillus niger on 10 per cent. glucose	1·17–1·19	Puriewitsch, 1900

[1] After an initial dark period.

presumption that a carbohydrate—any carbohydrate—is being quantitatively respired to carbon dioxide and water.

Where an R.Q. of unity, or some very close approximation, has been established it is reasonable to suspect the dependence of respiration upon a carbohydrate substrate. It is significant that it occurs commonly in adult leaves, the organs of photosynthesis. Mature barley leaves with a high carbohydrate content have been shown to have an R.Q. close to 1·0 and to be consuming sucrose.[1] Pal's investigation on *Spirogyra* showed an accurate R.Q. = 1·00 with simultaneous disappearance of starch; and a similar observation has been made during the rapid consumption of starch in aroid spadices. When the starch was exhausted, the R.Q. fell to 0·5 in *Sauromatum* and to $< 0{\cdot}87$ in *Arum*. Similar falls after the partial exhaustion of carbohydrates in detached leaves have already been mentioned (p. 48). Saprophytes, having little internal reserve, lend themselves to feeding experiments with known substrates. *Aspergillus* and *Phycomyces* on carbohydrate media gave R.Q.s close to 1·0 (Table 20). The same *Phycomyces* gave R.Q. = 0·66–0·75 on a linseed preparation. Some algae, as *Scenedesmus* (Table 20), respond similarly.

Fats, in general, give rise to R.Q.s around 0·7; many other substances, notably organic acids, give R.Q.s above one. It is therefore possible for values approximating 1·0 to arise by compensation between two oxidations. The R.Q. may also be raised by obstruction to the entry of oxygen, and a fatty respiration quotient thereby be made to simulate a carbohydrate one (see p. 89); even the most attractively unitary quotient is open to doubt until its bona fides is given collateral support. An important corollary is that where a quotient of 1·0 has been backed by evidence of carbohydrate consumption no considerable accumulation of intermediate oxidation products is likely.

The complete oxidation of proteins to carbon dioxide, water, and ammonia is able to give an R.Q. so close to 1·0 as to be experimentally indistinguishable from it. A virtually complete amino-acid analysis of edestin, the reserve protein of hemp seeds, is now available,[2] and leads to a calculated R.Q. = 0·991. There need be no confusion with carbohydrate utilization on this score, since the formation of free ammonia in the ratio $2NH_3$ to $7CO_2$ is also predicted. In the absence of ammonia formation, a unitary R.Q. cannot result from proteolysis. Protein breakdown has been shown to occur for a certain period in detached leaves in such a way as to involve oxidation without release of ammonia, amides accumulating instead. The oxidation of edestin to asparagine, carbon dioxide, and water would have an R.Q. = 0·743, or, substituting glutamine for asparagine, 0·797. The R.Q.s actually observed did not fall below 0·8 (p. 48). A later stage, in which the amides were fully oxidized with release of ammonia, should lead to R.Q.s rather above one (Table 21); and an approach to this also was observed (Fig. 13).

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[1] Yemm, 1934.

[2] See Chibnall, 1946.

TABLE 21

Calculated quotients for the respiration of a number of plant substances

Hexoses, hexose phosphates, and hexose anhydrides	1·000
Inositol and Phytin	1·000
Edestin (protein)[1] to ammonia	0·991
Edestin (protein) to asparagine	0·743
Trilinolenin (fat)	0·740
Tristearin (fat)	0·699
Malic and citric acids	1·333
Oxalic acid	4·000
Amino-acids—glycine	1·333
—leucine	0·800
—phenylalanine	0·900
—lysine	0·857
—aspartic acid	1·333
—glutamic acid	1·111
Amides—glutamine	1·111
—asparagine	1·333

Variation of R.Q. with temperature

It is frequently found that the value of the respiratory quotient alters when temperature is either raised or lowered. After a period of physical adjustment to the new conditions, a fresh value is established characteristic of the new temperature. The earliest experiments gave conflicting results;[2] but the available methods were not above suspicion. Puriewitsch (1905) endeavoured to improve technique and examined a very wide range of materials. He consistently found rising R.Q.s with temperatures increasing from just above zero to 35–37° C. Harrington (1923) examined the gaseous exchanges of dormant and just-germinating apple seeds at a range of temperatures running from 0 to 30° C. One series of experiments with dormant Newtown Pippin seeds, which may be taken as an example, gave mean ratios:

Temperature	0	10	13	19	30° C.
R.Q.	0·37	0·61	0·70	0·71	0·76

Harrington concluded that at 19° C. the R.Q. corresponded approximately to a complete oxidation of fats (cf. Table 21), but that it both rose and fell with temperature. The striking fall at 0 or even 10° C. can only be explained by what Harrington called a 'storage of oxygen', i.e. the formation of substances richer in oxygen than the fats themselves; the most probable are carbohydrates and perhaps, in smaller quantities, organic acids. Similar results have also been recorded for the acorns of two North American oak species[1] and the values ran even lower. For *Quercus alba* they were

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[1] Composition given in Chibnall, 1946.

[2] See, e.g. Déhérain and Moisson, 1874; Bonnier and Mangin, 1884; Gerber, 1896.

Temperature .	2·5	5	10	15	20	25	30° C.
R.Q. . . .	0·1636	0·1952	0·2977	0·3737	0·4673	0·6211	0·7127

and for *Quercus borealis* they were lower still. The R.Q. of germinating peas, on the other hand, did not vary between 0 and 50° C.[2]

The R.Q.s of green tomatoes also vary with temperature. Gustafson (1936) obtained a mean R.Q. = 0·78 at 15–18° C. and 0·98 at 30–33° C. The low R.Q. at 15–18° C. could not have been due to the oxidation of fats, because no fats were present. It was attributed to the accumulation of acids shown to be going on at this stage. Citric is the principal acid formed in tomatoes, and the observed R.Q. would be realized if one molecule of sugar were completely oxidized for every molecule converted to citric acid; but other acids are also found in green tomatoes.

The interest of these observations lies in their showing that temperature changes may alter the course of respiration as well as its rate. The thermal displacement of chemical equilibria would lead to the observed results. In the equilibrium, sugar $\rightleftharpoons$ citric acid, sugar formation is likely to be endothermic so that a rise of temperature will shift the equilibrium towards the left. If the sole reaction were sugar $\rightarrow$ citric acid, the R.Q. would be 0·0; if it lay in the opposite direction, the R.Q. would have no limit. The predominance of citric acid $\rightarrow$ sugar after a rise of temperature would, therefore, cause the R.Q. to rise also, its actual level being dependent on the proportion of sugar converted to CO_2 simultaneously. The same argument may be applied to the fat $\rightleftharpoons$ sugar equilibrium in acorns and apple seeds.

Variation of R.Q. with oxygen concentration

In one of the earlier investigations of R.Q., Stich (1891) examined the effect of reducing the concentration of oxygen available to wheat, maize, and pea seedlings. At 9–10 per cent. oxygen he found little change from the air value; but at about 3 per cent. oxygen a considerable rise occurred. He also showed that narcissus bulbs had R.Q.s close to 1·0 in air or 10·2 per cent. oxygen; but that in 7·5 per cent. oxygen the quotient had already risen to 2·36. The R.Q. of Granny Smith apples, investigated by Hackney (1943*a*), remained more or less constant around 1·0 down to 3 per cent. oxygen. Gustafson (1936) concluded that there was no appreciable change in the R.Q. of tomatoes until the oxygen concentration was lowered to 2 per cent. In one series of experiments with red tomatoes kept at 30–33° C. he found

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[1] J. W. Brown, 1939.

[2] Fernandes, 1923.

Oxygen per cent.	0·2	0·5	1·0	2·2	3·9	6·3	13·0	19·4
R.Q.	3·12	4·04	2·01	1·33	1·00	1·04	1·00	1·03

Similar series were observed with green tomatoes, and at 15–18° C. Carrot slices also show such rises and at somewhat higher oxygen concentrations.[1] Suspended in aerated water they gave R.Q. = 1·01 which was not altered appreciably by blowing pure oxygen in place of air. With 1·5 per cent. glucose in the medium, the R.Q.s rose a little, and the rise was made much greater by lowering the oxygen pressure.

Oxygen per cent.	0·5	2·5	5·0	21·0	100·0
R.Q.	4·8	2·5	2·4	3·06 1·70	1·08

Carrot disks rather readily form alcohol and, according to El Gawardi (unpublished), may even do so to some extent under air, causing a rise of the R.Q. to 1·13. This appears to be augmented with glucose in the medium. Similar rises have been observed in wheat roots.[2]

The simplest interpretation of these results is to suppose that anaerobic respiration, with formation of alcohol or other products, is not limited to complete absence of oxygen, but persists in low oxygen concentrations, and is gradually extinguished as the concentration rises. Its complete suppression would appear to need at least 3 per cent. oxygen and considerably more in some tissues.

This reasonable assumption is confirmed by the results of using oxidase inhibitors. These are substances that at high dilution react specifically with enzymes catalysing the transfer of electrons to atmospheric oxygen, the final reactions of aerobic respiration. The combination renders the enzyme inactive, so reducing oxygen consumption. Carbon dioxide production is not cut down in a corresponding degree and the R.Q. rises. The application of M/500 cyanide to seedling barley leaves[3] caused the R.Q. to rise from around 0·90 to about 1·13, and after removal of the cyanide the R.Q. fell sharply; control leaves without cyanide showed only much slighter deviations from 0·90 during the same period. Apples treated with HCN vapours or H_2S showed even greater rises of the R.Q., going sometimes above 2.[4] The action of the cyanide is not always limited to depression of O_2-uptake, there is often a simultaneous increase of CO_2-output; but this may be an indirect consequence of disconnecting the metabolic system from oxygen. Sodium azide, another oxidase poison, raised the R.Q. of oat embryos, 24 hours from soaking, from 0·8 to 1·5.[1] A probably analogous change occurs spontaneously in apples towards the end of a long storage life. The R.Q. has been observed to rise to more than 1·3,[2] and at the same period alcohol and acetaldehyde accumulate in the tissues.[3]

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[1] Turner, 1940.

[2] Lundegårdh and Burström, 1935.

[3] James and Hora, 1940.

[4] Thomas, 1947. See also Genevois, 1927 (pea seedlings); van Raalte, 1937 (root tips of beans); and Boswell, 1950 (potato and carrot).

DRIFT OF RESPIRATORY QUOTIENTS

In the collected works of de Saussure[4] there is an account of what may be the earliest attempts to determine the ratio of the gas exchange by quantitative eudiometry. He announced that seeds gave off carbon dioxide during germination in volumes nearly equal to the volumes of oxygen absorbed; but that green leaves absorbed oxygen at night in greater volumes than they gave off CO_2. As a result the leaves tended to reduce the amount of air in an enclosed space. These results are not wholly in accord with those that have been obtained since; but do agree in showing that, even under normal conditions, the R.Q. often departs from unity. There is, in fact, a drift of R.Q. with age analogous to the drift of respiration rate discussed in the last chapter. Table 22 sets out characteristic values taken by the quotient in different parts and developmental stages of the barley plant.

TABLE 22

Respiratory quotients of barley at 20° C.

Material	*R.Q.*	
Dormant grain	0·64	James and James, 1940
Soaked grain	1·55	,, ,, ,,
Submerged grain	7·50	,, ,, ,,
Embryos, 75 hours' germination	0·25	,, ,, ,,
Embryos + 6 per cent. sucrose	0·80	,, ,, ,,
Embryos + starchy endosperms	0·74	,, ,, ,,
Etiolated seedlings	0·95	,, ,, ,,
Seedlings starved 21 days	0·80	,, ,, ,,
Leaves forced in greenhouse	0·80	James and Norval, 1938
Mature leaves from complete sand cultures	1·03	McKee, 1937
Mature leaves from field	1·02	Yemm, 1934
Mature leaves darkened on plant	0·88	,, ,,
Mature leaves cut and starved 4 days	0·80–0·82	,, ,,
Young roots, tip cm.	0·94	Machlis, 1944*b*
Young roots, next 6 cm.	0·94–0·99	,, ,,

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[1] Albaum and Eichel, 1943.

[2] Thomas, 1947.

[3] Fidler, 1933*a*.

[4] *Recherches chimiques sur la Végétation*, 1804, chapters 1 and 3.

Leaves

Mature leaves usually have an R.Q. very close to 1·0 (Tables 20 and 22), and McKee (1937) found little variation in barley with alterations of nitrogen supply. The changes consequent upon detachment have been discussed on p. 48, and the lowering of the R.Q. in darkened and forced leaves are, no doubt, due to the same cause; i.e. to the replacement of carbohydrate substrates by proteins with consequent amide formation. A time drift is also evident in the R.Q. level of normal leaves on the plant. The unitary value is characteristic of mature leaves, and old leaves tend to show lower ones. Thus young *Syringa* leaves were found to have R.Q. = 0·95 and old ones an R.Q. = 0·83.[1] The falling value in old leaves is probably due to failure of their photosynthesis, again leading to shortage of carbohydrates.

Germinating seedlings

The behaviour of the R.Q. during germination has been studied intensively. It is now clear that de Saussure's statement can be applied only to a comparatively limited period; and the entire drift, from the moment of wetting the seed until the seedling is self-supporting, is long and rather complex. In later papers (1833–4) de Saussure amplified his results. Two experiments with wheat both gave R.Q. = 1·02, and other nineteenth-century investigators[2] also obtained values in the neighbourhood of 1·0. It was, however, realized that the values obtained in the earliest stages depended very much on the conditions of germination, and particularly on the method of wetting. Soaking by total immersion of the seeds causes very high initial R.Q.s, which slowly drop when the seeds are set out in air, or even if they are kept saturated. Peas have been a favourite object of study; Godlewski found that after 48 hours' soaking their R.Q. for the first 24 hours in air was 1·981 and for the second 24 hours, 1·296. Shorter periods of soaking give even higher R.Q.s for a while, and values up to 7·5 have been observed. The following data show how the R.Q. of peas falls with time:

After . .	4	8	24 hours' soaking[3]
R.Q. . .	6	1·8	> 1·0

It has been reported that there is an actual reduction in the rate of CO_2-emission during such a period, whereas peas allowed to take up water from a moist substratum show a rising CO_2-production from the very start,[4] even though their R.Q. may rise above 2·0, and only slowly descend towards 1·0 as the radicle emerges.[5] If the seeds are brought into oxygen instead of air after soaking, the R.Q. is lower, but still above 1·0 for some time.[1] Other seeds that have given similar results are mustard[2] and wheat.[3] Smith has pointed out that intercellular spaces are wholly or almost absent from the dried seeds. It may therefore be supposed that the seeds suffer a temporary lack of oxygen when they are sufficiently moistened to start up their respiration, but not wet enough to swell their cells fully. It is only when swelling is more or less complete that the cells will be rounded off and the intercellular spaces open to admit the passage of whatever oxygen is available.

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[1] Ullrich and Ruhland, 1928.

[2] e.g. Godlewski, 1882; Lehmann and Aichele, 1931, give a summary.

[3] A. J. M. Smith, 1935.

[4] Geiger, 1928.

[5] Fernandes, 1923.

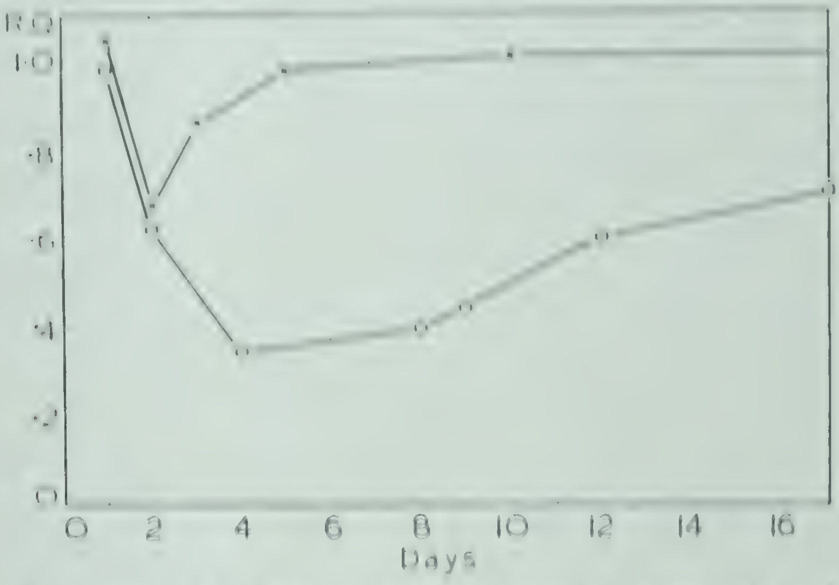

FIG. 29. Drift of R.Q. during the germination of, ×, wheat and, ○, flax seedlings. Data of Bonnier and Mangin, 1884.

From such results it seems clear that the R.Q.s observed in the first 24 hours or so of germination will depend much more on physical factors than upon the nature of the reserves consumed. Bonnier and Mangin (1884) tried to investigate the effect of substrate by comparing the R.Q. drifts of wheat (starch storing) and flax (fat storing). Their results (Fig. 29) show that there is indeed a wide difference, and that, in general, the fat-storing seed takes a lower course than the starch-storing grain, in accordance with expectation. Neither follows a simple course at the expected levels of 1·0 (starch) and 0·7 (fats), but each plunges to a lower level, and only slowly works back towards its ceiling. These results have since been amply confirmed. Stiles and Leach (1933) obtained results resembling those of wheat with maize, broad beans, and sweet peas, all starchy seeds, and others resembling parts of the flax drift with castor oil and sunflower seeds. Lupins, with their principal reserve in the form of hemicelluloses, behaved like starchy seeds. Two species gave less expected results. Vegetable-marrow seeds, though containing about 50 per cent. of fats and little carbohydrate, had R.Q.s that remained obstinately high, rarely falling outside the range 0·95–0·75. Buckwheat, on the other hand, although containing little fat, showed an R.Q. = 0·47, three and three-quarter hours after moistening, which rose steadily, reaching 0·96 after about 2 days.

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[1] Godlewski, 1882; A. J. M. Smith, 1935.

[2] A. J. M. Smith, 1935.

[3] Frietinger, 1927.

The respiratory quotients of dormant seeds appear to be low. Amongst fatty seeds, apple pips have been found to give R.Q. = 0·7[1] and acorns have given R.Q.s about 0·5 at 20° C., falling with the temperature.[2] Dormant barley grains, though mainly storing starch, have R.Q. = 0·64.[3] With the exception of buckwheat, all R.Q.s recorded for the earliest stages of germination start much higher than these figures and are more or less rapidly falling. It is evident that there must be a discontinuity in these records where dormancy is first broken; but results now available for barley grains fill the gap.[3] Fig. 30A plots the drift of R.Q. in dry grains placed on moist sand and shows clearly the sudden rise, reaching a peak after 4 hours, followed by an equally rapid fall and a slow recovery to about 0·95. During the early oscillation, the curve twice cuts the 1·0 level at an acute angle. It is impossible to suppose that these rapid changes represent as many transitions from one

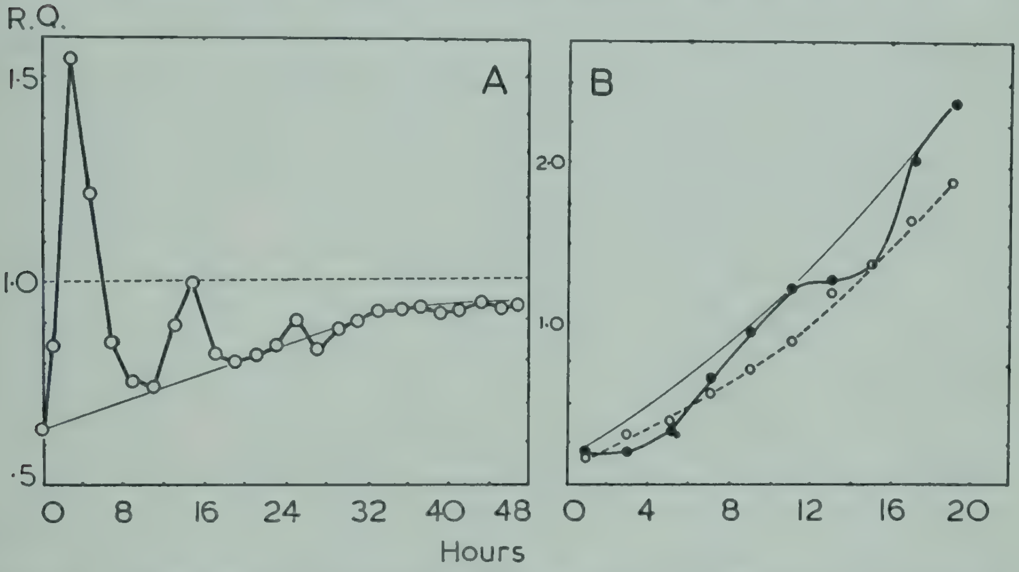

FIG. 30. A. Drift of R.Q. of barley seedlings. The thin line is drawn as a simple transition from dormant to steady values. B. CO_2-output ○– – – –○ and O_2-intake ●—●, in c.c. per 2 hours per 80 seedlings, from the same experiment. The thin continuous line shows what the O_2-uptake would be if the R.Q. followed the thin line in A. James and A. L. James, 1940.

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[1] Harrington, 1923.

[2] J. W. Brown, 1939.

[3] James and James, 1940; Merry and Goddard, 1941.

type of respiratory substrate to another, and fortunately a simpler explanation is to hand. The fluctuations of the R.Q. are not due to vagaries of CO_2-emission, which increases steadily throughout; but to irregularities in oxygen absorption (Fig. 30B). This shows that the sudden rise of R.Q. is not due to the sudden release of previously stored CO_2. Further, the effect can be greatly increased by obstructing the entry of oxygen by more liberally wetting the grain. Oxygen uptake then actually falls off and the R.Q. rises above 7·0 (Fig. 31A). The origin of the oxygen block is thus associated with the wetting of the grain and depends on the low solubility of oxygen in water. A film of moisture is formed over the surface of the grain even if it is not wholly submerged; but under such conditions the block is only transient and disappears as the colloids swell and growth starts (cf. p. 90).

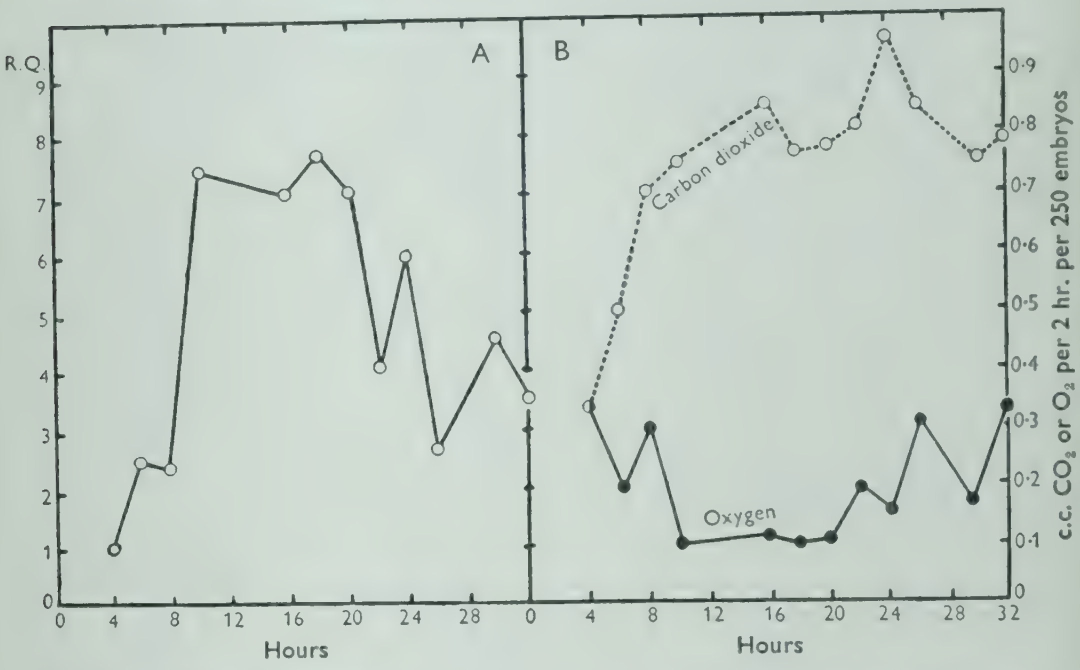

FIG. 31. Gas exchange of 250 detached embryos immersed in shallow water. A. Respiratory quotient. B. Carbon dioxide output, ○– – – –○ ; oxygen intake, ●—●. James and James, 1940.

Setting aside the physical complications, the chemical processes determining the drifts are qualitatively the same in both starchy and fatty seeds. Both types show low R.Q.s in dormancy and an early dip, followed by a later rise towards unity. The only difference is that in fatty seeds the dip is deeper and more prolonged. The buckwheat drift, recorded by Stiles and Leach, showed no initial up-kick but a steady rise throughout, and we may suppose that, were it possible to avoid the temporary oxygen-block, the other records would do the same.

In view of the low R.Q.s shown temporarily even by starchy seeds, it would seem likely that some utilization of fats occurs in them also. This is borne out by the fact that adequate quantities of fats have been determined analytically, and that they have been shown to diminish during dormancy and germination, especially in the embryo.[1]

TABLE 23

Percentage of ether-soluble material in dry matter of certain seeds

Starchy 'seeds'		
Zea mais	7·77	König, 1903
Triticum sativum	3·01	Thatcher, 1915
Fagopyrum esculentum	2·68	König, 1903
Hordeum sativum	2·32	James and James, 1940
Vicia faba	1·68	König, 1903
Hemicellulose seeds		
Tropaeolum majus	8·78	Rees (Stiles and Leach, 1933)
Lupinus luteus	4·25	König, 1903
Fatty seeds		
Ricinus communis	55·0	König, 1903
Helianthus annuus	54·1	Miller, 1912
Linum usitatissimum	35·5	Dillman, 1928

A complete respiration of the fats to carbon dioxide and water, even supposing no carbohydrate consumption occurred simultaneously, could not lead to R.Q.s lower than 0·7. The minimal R.Q.s actually observed are collected in Table 24, and it will be seen that all the fatty seeds, and even most of the carbohydrate-storing seeds, get below this

TABLE 24

Minimal R.Q.s during germination at 12–25° C.

Maize	0·73	Stiles and Leach, 1933
Wheat	0·61	Bonnier and Mangin, 1884
Buckwheat	0·47	Stiles and Leach, 1933
Barley (whole grain)	0·74	James and James, 1940
,, (embryo)	0·23	James and Norval, 1938
Broad Bean	0·35	Bonnier and Mangin, 1884
Sweet Pea	0·85	Stiles and Leach, 1933
Tropaeolum majus	0·70	,, ,, ,,
Lupinus luteus	0·76	,, ,, ,,
,, ,,	0·42	Bonnier and Mangin, 1884
Sunflower	0·55	Stiles and Leach, 1933
Flax	0·30	Bonnier and Mangin, 1884
Castor Oil	0·495	Stiles and Leach, 1933
,, ,,	0·30	Murlin, 1933
Cress	0·35	Bonnier and Mangin, 1884
Vegetable Marrow	0·64	Stiles and Leach, 1933
Mustard	0·49	Knabe, 1942

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[1] Yocum, 1925 (wheat); Toole, 1924 (maize); James and James, 1940 (barley).

level. Most of the fat in the barley grain is in the embryo, and, when detached from the endosperm, this reaches the lowest R.Q. yet recorded at normal temperatures. An interesting analogy is Pal's measurement of R.Q. = 0·27 in conjugating *Spirogyra*, supported by the observation that fats are present at this stage, and that the starch also present does not disappear in spite of the enhanced oxygen uptake. It has been shown with several fatty seeds that, at the stage when the R.Q. is known to be at its lowest, the fats are disappearing; but starch and sugars are being formed.[1] Knabe (1942) measured the simultaneous consumption of oxygen and fats during an 8-day germination of mustard seedlings, and compared them with the CO_2-output. Assuming that the oxidation of the fats could be approximated by the equation,

$$2CH_2+3O_2 = 2CO_2+2H_2O$$

he calculated the excesses of fat and oxygen consumptions over CO_2-production for the mean result of four experiments. Approximate unity was found for the ratio CH_2/O, corresponding with the equation $CH_2+O = CH_2O$, i.e. the conversion of fat to sugar. The mechanism of the conversion is obscure, but is believed to proceed via hydrolysis with subsequent breakage of the fatty acids into smaller and more oxidized chains.[2] This could occur without release of CO_2 to give R.Q. = 0. The greater the proportion of fat being oxidized to sugars and the smaller the proportion being oxidized to carbon dioxide and water, the farther will the R.Q. fall below 0·7. The observed R.Q.s and the analytical data are in agreement that the conversion of fat to sugars is active during the germination of fatty seeds. The R.Q.s indicate that this must also be going on in the hemicellulose and starch seeds. This is particularly clear in the isolated barley embryos (Table 24 and Fig. 32), which contain most of the grains' fats and in which starch, though absent in dormancy, appears immediately germination begins.[3] This cannot, however, be the whole of the story. When the embryos develop their extremely low R.Q.s, carbohydrates are being lost as well as fats,[4] and incomplete carbohydrate oxidations, such as the formation of pectic substances in the middle lamella, are likely to contribute to a depression of the R.Q. A possible product of fat oxidation is cutin,[5] which may be regarded as a complex oxidation and condensation product of fatty acids. Although cutin dissolves fat-soluble dyes, it is itself no longer soluble in ether. It has been shown that the coleoptile of barley grains and the first leaf rolled within it have a well-developed cuticle, which is rapidly increasing its area; so there can be little doubt that cutin is being formed. Its synthesis thus seems likely to contribute to the depression of the R.Q. when fats are being oxidized.

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[1] Detmer, 1880 (hemp); Leclerc du Sablon, 1893–7 (*Ricinus communis*); Miller, 1910 (sunflower); see also Miller, 1938, for further citations.

[2] Matthes, 1927.

[3] Brown and Morris, 1890.

[4] A. L. James, 1940; James and James, 1940.

[5] Priestley, 1924.

After the initial period of fat consumption, carbohydrates contribute a progressively increasing proportion of the respiratory substrate. Indeed, it seems probable that during germination there are never pure fat or pure carbohydrate phases, but only a change of proportions. In starchy seeds there is a carbohydrate contribution from the first, which progressively and rapidly supersedes the small fat component; in fatty seeds there is a preponderance of fat respiration at the start which gradually gives way to an increasing carbohydrate contribution as the fats are respired or converted to sugars. It is interesting to note in this connexion that the minimal R.Q. for complete barley grains, which is 0·74, does not indicate a straight respiration of fats to carbon dioxide and water, but is the resultant of collateral processes: partial oxidations of fats to carbohydrates and cutin, of carbohydrates to pectins, and full oxidation of carbohydrates to carbon dioxide and water. Whether there is also complete oxidation of fats to carbon dioxide and water without prior conversion to sugars is at present unknown. That the partial oxidation of fats and complete oxidation of carbohydrates are additive is shown by the results of feeding sugars to the isolated embryos. The

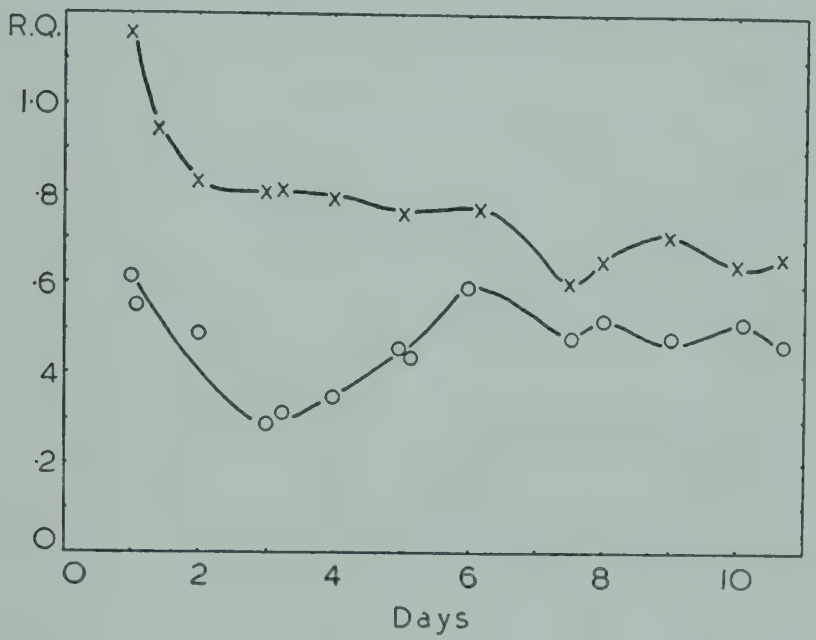

FIG. 32. Drift of R.Q. in detached barley embryos. ×, embryos with sucrose; ○, without sucrose. Data of A. L. James, 1937.

rate of carbon dioxide production is increased and the R.Q. rises to values intermediate between those of the unfed embryo and 1·0.

CARBON DIOXIDE FIXATION

Some of the most sensational fluctuations of R.Q. are those recorded for succulent leaves during periods of darkening following carbohydrate accumulation in light. At such times, Crassulacean leaves show a rapid accumulation of di- and tricarboxylic acids accompanied by reduced CO$_2$-production and lowering of the R.Q. In favourable circumstances it has even been observed that the R.Q. becomes negative, and that

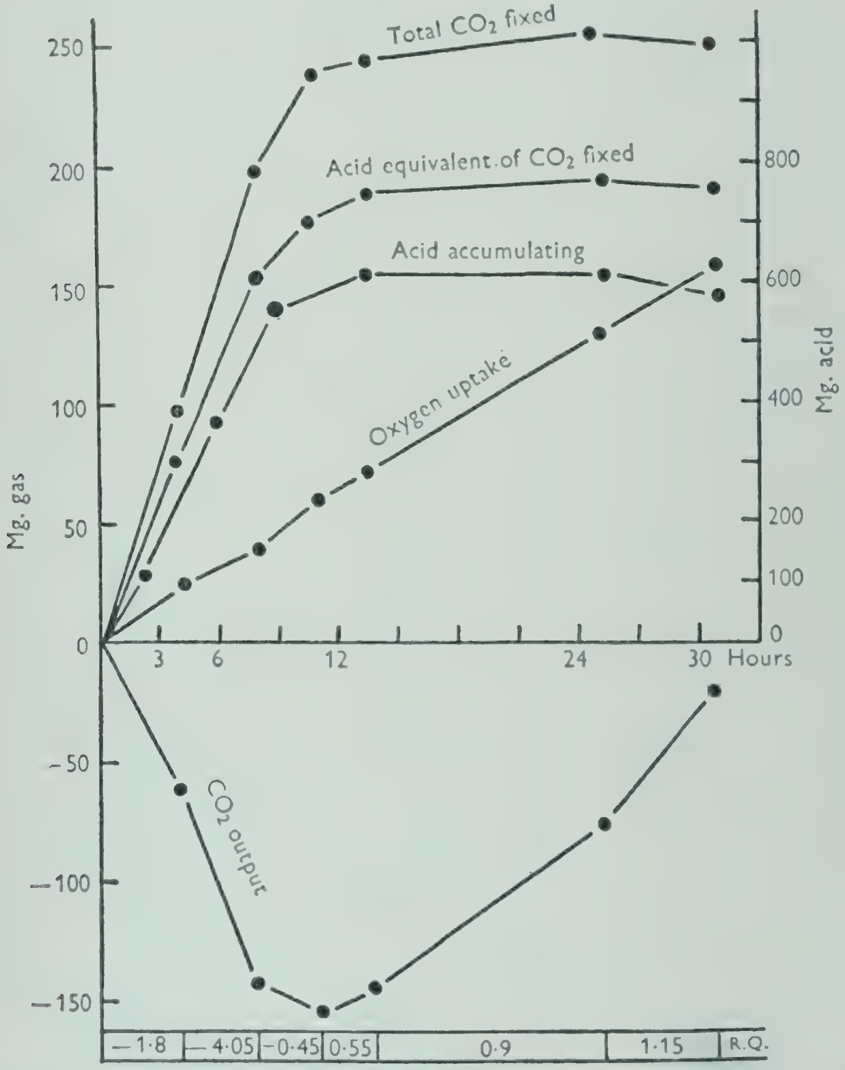

Fig. 33. Gaseous exchanges and accumulation of titratable acids in *Bryophyllum* leaves kept in the dark at 12° C. in a gas mixture initially containing 5 per cent. CO$_2$. After Thomas, 1951.

CO_2 of respiration and CO_2 in the external atmosphere may be absorbed (Fig. 33). The phenomenon is apparently most obvious in *Bryophyllum* leaves[1] and the optimal conditions are defined as 2–5 per cent. CO_2 at a temperature of 12° C. or lower,[2] with leaves initially rich in carbohydrate and poor in acid. The period of rapid acidification is then characterized by rapid CO_2-absorption and negative R.Q.s. As acid formation levels off, CO_2-intake ceases, and the negative R.Q. steadily decreases, finally becoming positive, and rising above unity when acids begin to diminish. Throughout the entire sequence, the rate of oxygen-uptake remains constant. There is thus a strong presumption that acid formation in *Bryophyllum* and related species involves the consumption of free CO_2. The observed simultaneous CO_2-disappearance cannot be attributed to solution in cell saps because it diminished at concentrations above about 5 per cent. external CO_2. Application of $^{14}CO_2$ to *Bryophyllum* leaves has shown that at 2–3° C. a considerable fraction of the labelled carbon was incorporated in organic acids after 60 hours, but that detectable amounts were also present in carbohydrates, amino-acids, and even proteins.[3] Although it is easiest to demonstrate in the dark, there is good reason to suppose that the phenomenon persists in daylight. The mechanism of the CO_2-fixation reaction in higher plants has been described on p. 37.

It is rarely possible to secure conclusive results in metabolic studies by the application of a single method. It is only when several independent lines of approach have led to a common result that real confidence can be felt; and it is in this light that the determination of R.Q.s must be viewed. As an exploratory technique it has great value owing to its ease; and, in addition, its relatively high order of accuracy makes it sensitive beyond the range of most biochemical methods. It has thus proved a safeguard against too hasty conclusions from analytical data and a means of revealing directions of metabolic change that might otherwise have escaped notice.

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[1] Thomas and Beevers, 1949; Thomas, 1951.

[2] See p. 36 for the effect of temperature on sugar–acid equilibria.

[3] Thurlow and Bonner, 1948.

V

RESPIRABLE MATERIALS

The evidence of the drift and R.Q. experiments described in the two preceding chapters indicates the carbohydrates as the principal group of respirable substances in higher plants. Fats may replace them during the germination of seeds, and proteins during metabolic degradation; but the normal support of the healthy vegetative tissue is a carbohydrate of one sort or another. In support of this we may point to the universal presence of carbohydrates in plant tissues, their disappearance during active respiration, and the frequent occurrence of unit R.Q.s. In addition there is the close relationship, under favourable conditions, between certain sugar concentrations and the rate of respiration, and the positive results of feeding sugars to respiring tissues.

FEEDING EXPERIMENTS

The further investigation of the carbohydrate basis of respiration has been carried on mainly by two methods—feeding and analysis. The general principle of the feeding method is simple; the tissue is first starved and the desired substance afterwards supplied in solution. The results observed are not always so simple, and it is necessary to realize that positive results are only to be expected if the solute is able to pass the tissue membranes at a fast enough rate, if its internal concentration has been reduced enough beforehand, if all other respiratory requirements are capable of sustaining an increased rate, i.e. if the starvation has not removed other essentials as well as the one in mind; and provided the abnormal method of application does not result in abnormal reactions. It is clear that negative results have little significance. On the other hand positive results do not necessarily prove that the reaction normally exists in the tissue; but only that it can exist given the necessary provocation. The results of feeding experiments are therefore to be regarded as a link in a chain of evidence, not as binding in themselves.

Feeding experiments with leaves

Palladin (1901) floated etiolated broad bean leaves with dipping petioles on a 5 per cent. solution of sucrose. After two days the leaves were transferred to water and gave off CO_2 at the rate of 109·1 mg. per hr. per 100 gm. F.W. Prior to the sucrose feeding the figure had been 40·1 mg. Comparing different sugars supplied in the dark, he found glucose better than sucrose; but maltose and raffinose poorer. Later[1] he compared different concentrations and found an optimum for increased CO_2-production at about 5 per cent. Maige and Nicolas (1910) followed up these observations using etiolated broad bean shoots cut from seedlings germinating from 2 to 3 weeks in the dark. They obtained increased CO_2-production up to about 20 per cent.; but reduction at 30 per cent. They found little difference between sucrose, glucose, and fructose; lactose gave no increases. The respiratory quotients of the starved shoots were very low, usually close to 0·5. Increasing the sugar concentrations raised them progressively, the highest R.Q. observed being 0·92 with 30 per cent. glucose. Unfortunately, they give no control figures without glucose, so the R.Q. of the additional gas exchange due to the sugar itself cannot be calculated. In other experiments where controls are given, the R.Q.s of the additional respiration are very irregular. Similar results were obtained with seedlings of kidney beans, starved by removal of their cotyledons and being kept in the dark, except that their optimal sugar concentration lay nearer 10 than 20 per cent. Spoehr and McGee (1923) used cut sunflower leaves that had not previously been starved. When darkened with their petioles dipping into a mineral nutrient solution at 24° C., they showed a rapidly falling respiration rate, which was about one-third the original after 3 days. When 7 per cent. sugar was added to the medium, the fall was retarded or abolished. The initial rates also appear to have been higher; but as controls were not run simultaneously this is open to some doubt. Increased CO_2-production was observed with sucrose, glucose, and fructose. With 7 per cent. mannose the respiration rate began to rise rapidly after 10 hours. The authors express some surprise that mannose did not appear poisonous, as Knudson (1917) had reported its toxic action on root tips of wheat and peas. It is possible, however, that the sudden acceleration of respiration, not caused by any of the other sugars, was itself traumatic.

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[1] Palladin and Komleff, 1902.

A possible objection to the experiments of Spoehr and McGee is that their sugar solutions, although initially sterilized, may have become contaminated during the run of the experiments which lasted about 3 days. Ranjan[1] used a technique which made contamination much less likely. He kept detached cherry-laurel leaves on water until their respiration rate was slow and more or less constant. He then weighed the leaves and vacuum infiltrated them with sugar solutions, afterwards drying the surfaces and weighing again to obtain the amount of solution injected. Leaves injected with sugar solutions showed additional CO_2-production over those injected with water. The effect was temporary and all returned after varying times to the same rate of CO_2-emission. Positive and closely similar results were obtained with sucrose, glucose, and fructose; maltose and lactose had little effect and mannose proved strongly toxic. The amount of extra CO_2-production due to any of the first three sugars increased until the internal sugar concentration was raised to about 1·5 per cent. of the leaf fresh weight; further increases to 4 per cent. made no difference. Of the sugar injected, one-tenth to one-third was recovered as additional CO_2 by the time the output returned to normal, the remainder apparently being built into the leaf reserves.

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[1] Ranjan, unpublished.

Embryos

The embryos of cereal grains are particularly suitable for feeding. After an hour's soaking, they may readily be eased off their endosperms without the rupture of any living cells. The scutellum thus exposed is a tissue specialized for the absorption of organic compounds from the endosperm and therefore particularly well adapted to absorb sugar solutions from outside. Removal of the endosperm also establishes the necessary degree of starvation, since the embryo exhausts its own internal sugar supplies in about 24 hours. Brown and Morris (1890) detached barley embryos, and grew them on glass wool moistened with a variety of solutions. The embryos showed increases of dry weight after growing for 7 days on 3·5 per cent. solutions of sucrose, glucose, fructose, maltose, invert sugar, lactose, and raffinose. With sucroset he weight was more than doubled—89 mg. to 195·5 for 50 embryos—and with raffinose the increase to 91 mg. was slight and probably not significant. The other sugars produced intermediate values in the order given, and on water alone there was a drop to 52 mg. The effect of the sucrose feeding on the respiration has also been examined.[1] Precautions were taken to avoid the intrusion of moulds, yeasts, and bacteria and the solutions supplied in a substratum of sterilized sand. With mineral nutrients alone, the embryos slowly emitted CO_2 at the rate of about 37 μl. per hr. per embryo for 9 days, after which the plantlets collapsed. Addition of 2, 4, or 6 per cent. sucrose caused a rapid rise to a rate almost exactly three times as fast, which was maintained for at least as long, afterwards showing a slow decline. There was a slightly greater CO_2-output with 4 per cent. sucrose than with 2 per cent.; but further increase to 6 per cent. was without effect (Fig. 23, p. 69).

Other tissues

Sustained increases of respiration rates have been observed by feeding 2 per cent. sucrose to detached maize roots[2] and 1 per cent. sucrose to radish disks[3] and segments of oat coleoptiles.[4] Coleoptiles that had been separated from their endosperms for 96 hours showed 157 per cent. increase in the rate of oxygen consumption, whereas those still attached to endosperms showed only 22 per cent. rise. Positive results of glucose feeding have been observed in carrot slices,[1] sections of tomato stems,[2] and numerous unicellular algae.[3]

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[1] James and James, 1940.

[2] Girton, see p. 62.

[3] Saïd and El Shishiny, 1944.

[4] Bonner, 1948.

A certain consistency is observable in all these results of feeding experiments by independent investigators using the most diverse materials. Moderate concentrations—up to 10 per cent. or sometimes higher—of sucrose, glucose, or fructose, lead to increased respiration rates. Higher concentrations may cause reductions probably owing to osmotic complications. Other sugars, such as maltose, lactose, or raffinose, have little effect, and mannose is likely to be toxic. Feeding sucrose, glucose, or fructose to starved tissues with low R.Q.s tends to raise the value towards unity; but so far results are not available to show whether the additional respiration has a R.Q. = 1·0. This would be useful in showing whether the absorbed sugars are respired, or merely become the occasion of further internal breakdowns.

It cannot be assumed from the negative results with maltose, lactose, and raffinose that they are non-respirable. Definite evidence of the breakdown of internal maltose and raffinose is available (see p. 104). Sucrose appears to hold a special position; there is no evidence that it enters the tissues unchanged, but a good deal that it is broken down. Mrs. Onslow[4] found that sucrose injected into the intercellular spaces of cherry-laurel leaves, as in Ranjan's experiments, had given rise to glucose and fructose within 2 hours. Similar hydrolysis appears to occur at the cut surfaces of carrot and radish disks,[5] at the absorbing zone of wheat and rye roots[6] and in various leaves.[7] A good deal of evidence has been provided that the absorption of sucrose by tomato roots involves its prior phosphorylation. It has been estimated[8] that, from each pair of sucrose molecules decomposed, one glucose unit is absorbed as glucose-1-phosphate, while 1 glucose unit and 2 fructose units remain outside. This root absorbs the hexoses only very slowly.

ANALYSIS OF CARBOHYDRATES

An important result of carbohydrate analysis in respiring tissues has already been detailed in connexion with respiratory drifts. It was shown (pp. 45 et seq.) that, in the initial phases after detachment, the loss of labile carbohydrates was equivalent to the total CO_2-output by leaves of vine, broad beans, and wheat and to within 10 per cent. in barley. The loss of soluble sugars by detached maize roots has similarly been shown to be closely similar to the simultaneous CO_2-emission (p. 62). Apples in store, that have reached a stage where sucrose is disappearing, show a total sugar loss equal to the CO_2-emission.[1] All these except the maize roots are matured tissues without means of carbohydrate synthesis or capacity for growth. Where the latter exists, labile carbohydrate loss is found to exceed CO_2-production, owing to the transfer of sugar units to structural carbohydrates and other synthetic products.[2]

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[1] J. S. Turner, 1938*b*, 1940.

[2] Caldwell and Meiklejohn, 1937*a*.

[3] Genevois, 1929*b*; Taylor, 1950.

[4] Unpublished.

[5] El Gawadi, 1935; Saïd, 1941.

[6] Burström, 1941.

[7] Saïd, 1948.

[8] Dormer and Street, 1949; Street and Lowe, 1950.

The results of these analyses support the R.Q. data in suggesting that in mature tissues carbohydrates foot the whole of the respiratory bill. The analytical method is capable of distinguishing further between the individual carbohydrates concerned. In spite of technical difficulties, which are great, detailed results are now available for a number of tissues.

Analysis of leaves

Among the first tissues to be investigated were the vine leaves of Deleano. Starch, sucrose, and total hexoses were estimated. The two first rapidly disappeared, starch being completely gone by 100 hours and sucrose soon after. The hexoses showed little diminution during this time, and only suffered considerable losses when the carbohydrates were no longer accounting for the whole of the CO_2-output (Fig. 12). Similar results were obtained for cherry-laurel[3] but with different proportions of the various carbohydrates. Starch disappeared steadily, being wholly gone after 10 days. Sucrose was lost more slowly, while total hexoses maintained a fluctuating level around 1 per cent. of the leaf fresh weight, and were still present when the experiment was terminated. For the first 6 days, hexoses were below starch and sucrose in concentration, whereas in vine leaves they were always considerably in excess. Further results of the same sort, but at much faster tempo, are recorded for broad bean leaves (Fig. 34)[4] Starch is gone after 48 hours and sucrose much reduced. Glucose and fructose being estimated separately both showed an initial increase, followed by a slower decline. The form of their drifts suggests strongly that part of the starch or sucrose is converted into hexoses which are afterwards respired. The same interpretation could be placed on the previous results.

The most detailed investigation of the relation of leaf carbohydrates to respiration is Yemm's (1935) examination of barley leaves. In this leaf, sucrose is the principal reserve with the addition of some fructosan and starch in favourable seasons. Results for starchless and starch-forming seasons are shown in Figs. 35 and 36. General agreement is shown with the preceding. Sucrose and starch, when present, show immediate losses which result in the disappearance of starch after 100 hours and the almost complete disappearance of sucrose. This is confirmed by later observations with ^{14}C-labelled sucrose generated by photosynthesis, which, in addition, revealed a lag of at least 1 hour before loss of sucrose was detectable.[1] Glucose also behaves as before, showing first an increase and then a fall more or less parallel with the sucrose. Fructose, on the other hand, quickly disappears with no initial increment and no indication of its formation by the hydrolysis of the fructosans or other anhydrides.[2]

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[1] Onslow *et al.*, 1931.

[2] See, for example, germinating seeds, p. 65.

[3] Mrs. Onslow, unpublished.

[4] Yemm, 1934.

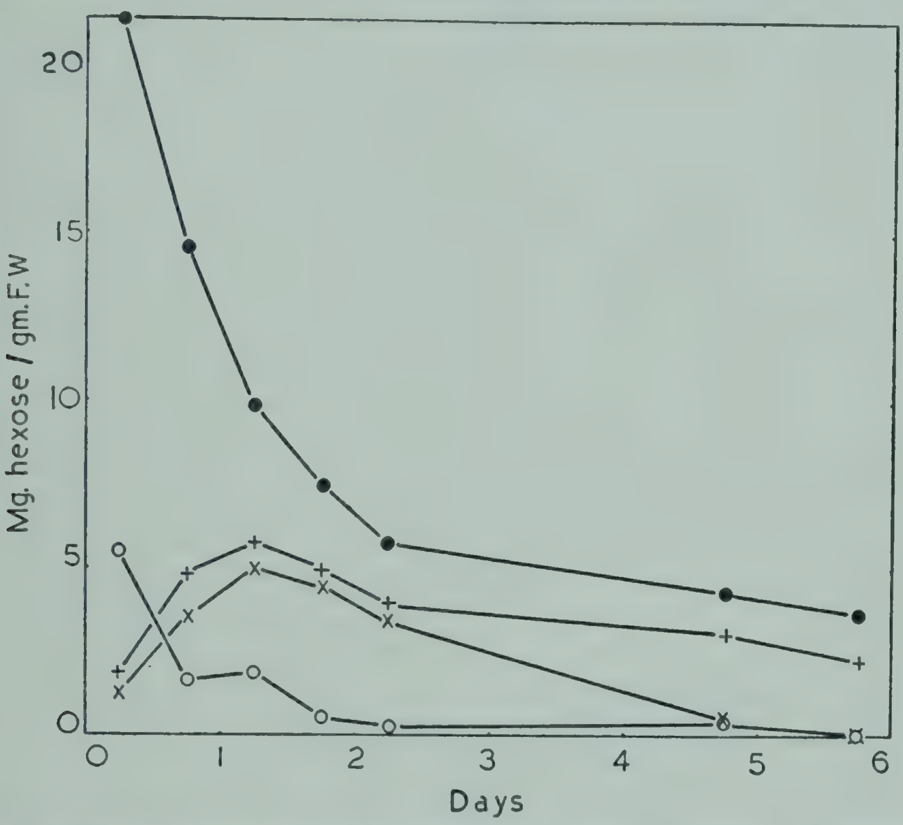

FIG. 34. Available carbohydrates in broad bean leaves. ○, starch; ●, sucrose; +, glucose; ×, fructose. After Yemm, 1934.

Seedlings

Corresponding data for barley seedlings during germination are provided by A. L. James (1940). About 90 per cent. of the CO_2-emission of the sprouting grain comes from the embryo,[1] and it is the carbohydrate changes in its tissues that are of the most immediate concern to respiration. Ultimately it depends on the hydrolysis of starch stored in the endosperm; but there is a time lag of about a day (Fig. 37A) before this is mobilized to any measurable extent.[2] It then breaks down with the formation of dextrins and sucrose, the latter probably providing the translocation form into the embryo. Changes of the carbohydrate fractions in the embryo itself are shown in Fig. 37B. Before supplies are received from the endosperm, the labile carbohydrates stored in the embryo itself are broken down. These are sucrose and raffinose, and both are exhausted by the end of the second day. Although no starch is present, maltose appears; but the hexoses are barely detectable. Owing to the active growth at this stage, two further carbohydrate fractions have to be brought into the account, cellulose and hemicellulose, and both increase, the latter largely. Although supplemented by the fats, sucrose and raffinose have completely disappeared by the end of the second day (cf. balance sheets, p. 65). On the third day, translocation from the endosperm being well established,

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[1] Calvin *et al.*, 1951.

[2] See also Wood, Cruickshank, and Kuchel, 1943; Wood, Mercer, and Pedlow, 1944 (Australian grasses); Krotkov, 1939 (wheat).

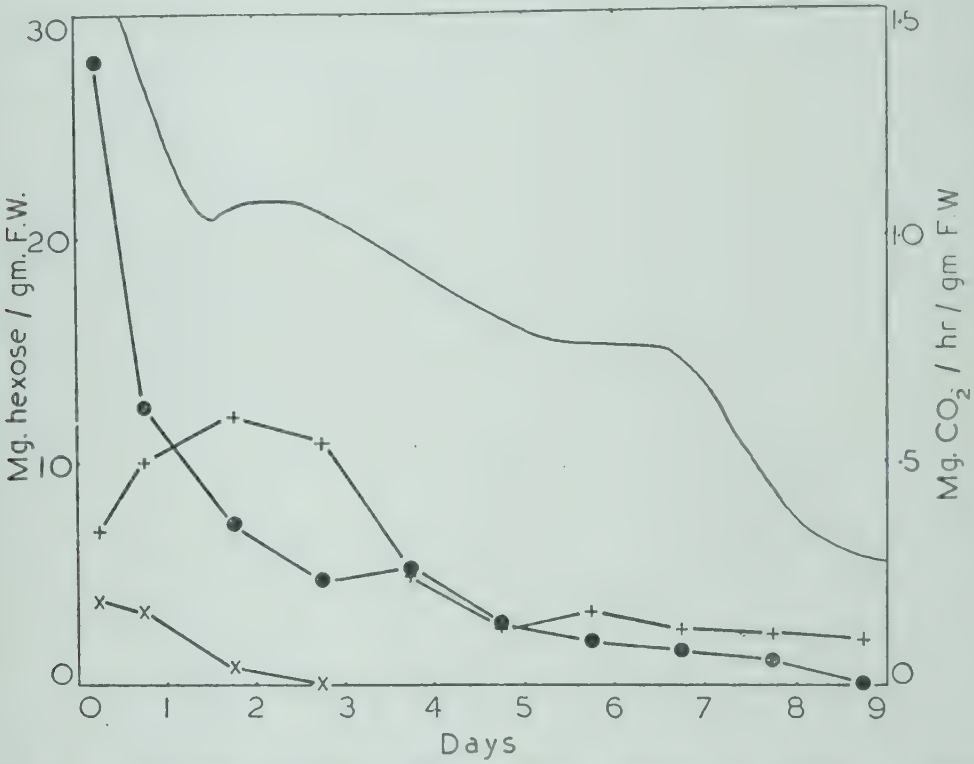

Fig. 35. Available carbohydrates in barley leaves. ●, sucrose; +, glucose; ×, fructose. The continuous line shows the rate of CO_2-emission. Data of Yemm, 1934.

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[1] Barnell, 1937; Bruschi, 1908.

[2] Hora, 1936.

sucrose concentration begins to rise rapidly, and the rise is maintained until exhaustion of the endosperm's starch begins to cut down translocation. Then the story of the first two days is repeated on a larger scale. Sucrose starts to disappear again, while maltose, the hexoses, cellulose, and hemicellulose all increase. Only in extreme starvation, when sucrose has virtually disappeared, is there any net loss of these materials. Embryos raised without access to endospermic or other carbohydrate sources show the same effects in an accelerated form and without any increase of sucrose concentration.[1]

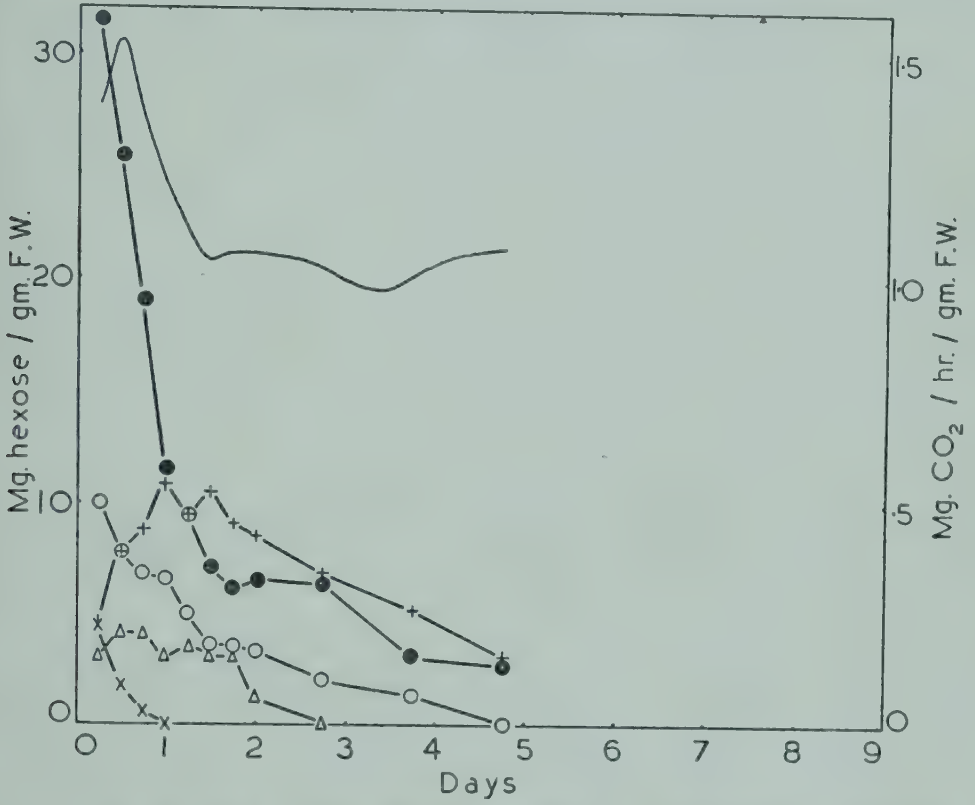

FIG. 36. Carbohydrates in barley leaves. ○, starch; △, fructoside; ●, sucrose; +, glucose; ×, fructose. The continuous line shows the rate of CO_2-emission. Data of Yemm, 1934.

Analysis of fleshy fruits

During the development of bulky fruits, carbohydrates are accumulated in the flesh in large quantities. Throughout this period all the main categories are increasing their concentration as well as their absolute amounts. Archbold's (1932) analyses of Bramley's Seedlings showed a steady increase of sucrose, glucose, and fructose, as percentages of fresh weight, and an accumulation of starch beginning about a month later in June. Towards the end of August, although the sucrose and hexose concentrations were still increasing, starch began to disappear. Sucrose loss was delayed until starch had almost entirely gone; and the hexoses persisted as before. The later stages leading to the climacteric are described from apples in store. After picking, there is a prolonged phase of sucrose loss interrupted at the climacteric by a temporary increase.[1] The phase of sucrose loss after the total disappearance of starch and before the climacteric rise is also a phase of slowly falling respiration rate, and represents a relatively simple relation between carbohydrate consumption and CO_2-production. Mrs. Onslow in 1931 assembled the data then available for a number of fruits during storage. Of a considerable number of analyses of apples and pears, almost all showed a gain of hexoses and a loss of sucrose. In bananas kept 25 days in store, there was a steady loss of starch until it was all gone at 21 days, and an accumulation of sucrose for about 12 days, followed by a steady

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[1] See Yocum, 1925, for similar results with germinating wheat, and Lehmann and Aichele, 1931, for a summary of earlier results with various cereals.

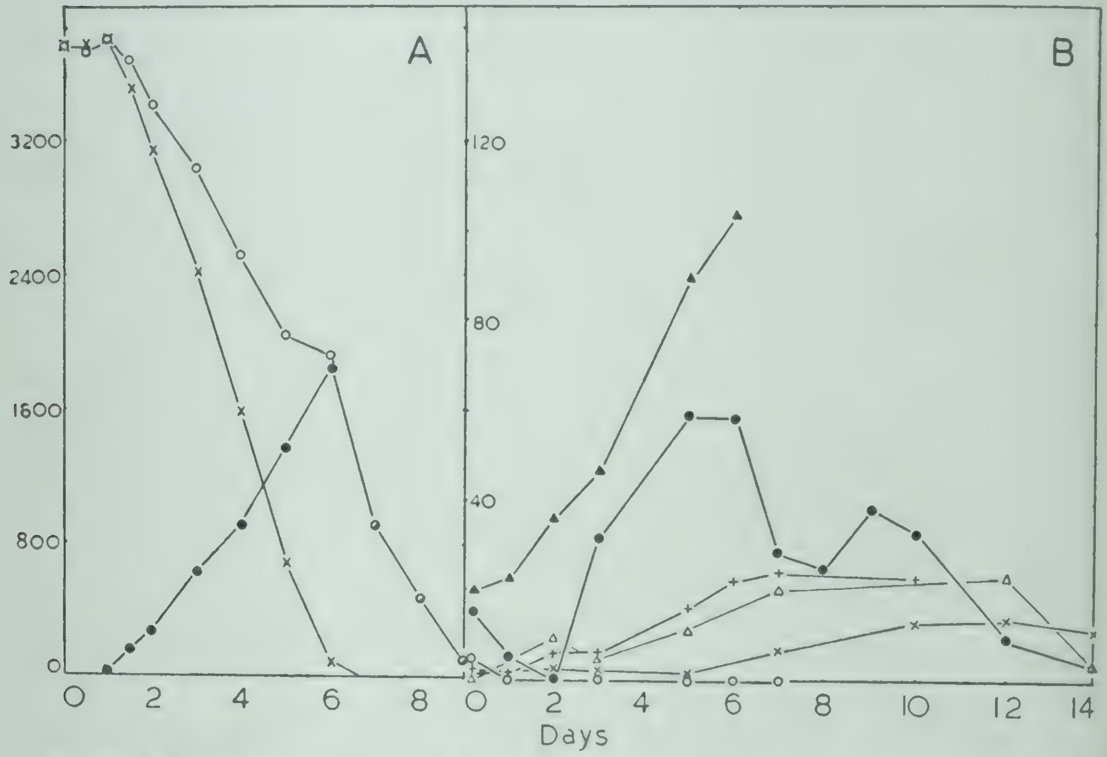

FIG. 37. A. Mobilization of starch in barley endosperms. ○, total labile polysaccharide; ×, starch; ●, dextrins. After Hora, 1936. B. Carbohydrate fractions in the embryos of germinating barley grains. +, cellulose; ▲, hemicellulose; ○, raffinose; ●, sucrose; △, maltose; ×, hexose. After A. L. James, 1940. The ordinates in both parts of the figure are mg. hexose equivalent per 100 grains.

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[1] Kidd and West, 1935.

drop for the rest of the period; hexoses accumulated throughout. Mrs. Onslow noted in these results a tendency towards approximate equality between the hexose accumulating and the hexose equivalents of the total sugar loss and one-half the sucrose loss. To examine this equivalence further, analyses were performed on starch-free Bramley's Seedling apples stored at 5° C.[1] Over a period of 150 days there was a steady loss of sucrose and gain of hexose. The carbon dioxide output was measured over the same period, and a correction applied for the observed loss of malic acid. The residual CO_2-output was equivalent to the total sugar loss and to one-half the sucrose loss. Apples at 15° C. at first containing starch showed the same relationships once the starch had disappeared. The hexose that accumulated was, in both experiments, about half glucose and half fructose (Fig. 38). The simple 2/1 ratio between sucrose and total sugars lost was a fortunate circumstance of these experiments rather than a general rule. The same authors report a wide range of values in similar circumstances. The significant fact of apparently wide application is the temporary increase of hexose during sucrose consumption. It has also been recorded for stored apples by Archbold (1932), who found that the increase was mostly accounted

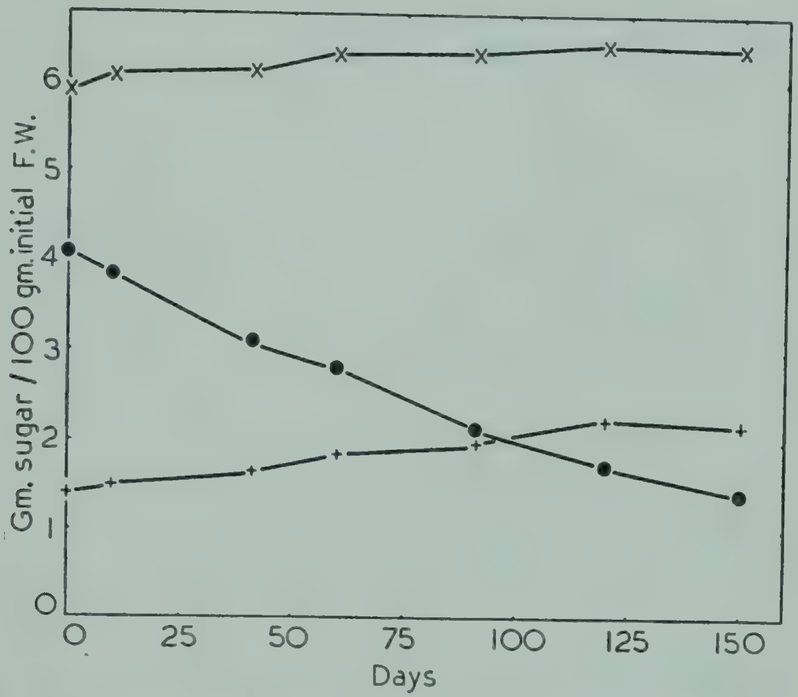

FIG. 38. Sugars in starch-free Bramley's Seedling apples stored at 5° C. ●, sucrose; +, glucose; ×, fructose. Data of Onslow, Kidd, and West, 1931.

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[1] Onslow, Kidd, and West, 1931.

for by fructose in Bramley's Seedlings and wholly in Worcester Pearmains. She observed further that when, later, the hexoses began to diminish it was fructose that went first. The hemicelluloses present are not broken down.[1]

In none of the foregoing results is there a close connexion between the rate of respiration and the concentration of any individual carbohydrate. The nearest approach is with the concentration of sucrose.

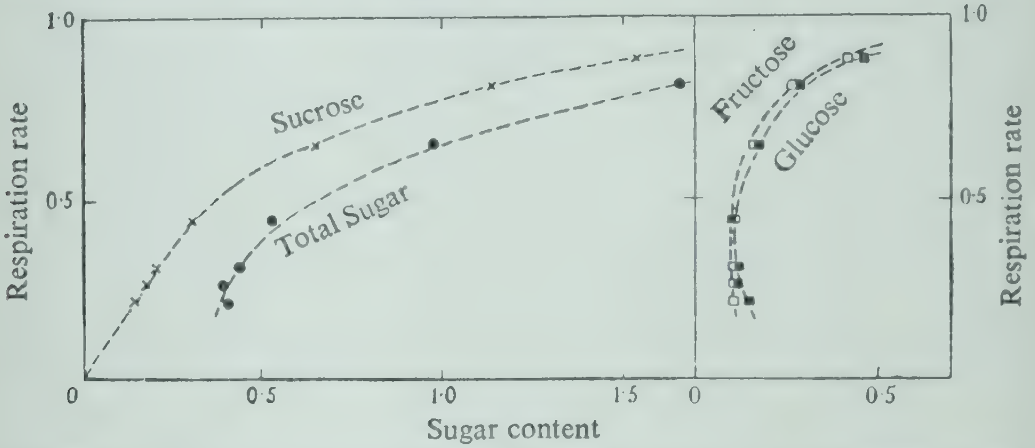

FIG. 39. Rate of CO_2-production of potatoes plotted against sugar concentration during sweetening at +1° C. ×, sucrose; ●, total sugar; □, fructose; ■, glucose. After Barker, 1936.

Falling respiration rates are associated with falling sucrose concentrations during the early phases of leaf starvation, after the disappearance of starch from barley grains, and during the early maturation of apples. Rising respiration rates are associated with increasing sucrose concentrations in the second phase of barley germination and, temporarily, in cooling potatoes. It would seem that, under favourable conditions, sucrose concentration might limit respiration rate. The possibility has been most thoroughly investigated in potatoes, where the temperature responses of the starch ⇌ sugar equilibrium provide a means of controlling the internal sugar concentration experimentally (see p. 33). This has been most extensively undertaken by Barker. Initial studies (1933) led to the conclusion that the relation between respiration rate and total sugar concentration was that of a rectangular hyperbola. Further investigation (1936) showed that the effective sugar in determining the relation was sucrose, and that the hexose sugars showed no constant relation with respiration rate (Fig. 39). In these experiments only rates not considered to be affected by low-temperature inhibition (p. 35) were taken into account. The goodness of fit to the rectangular hyperbola is illustrated for four experiments in Fig. 40 by plotting $1/R$ against $1/S$. At low concentrations, the hyperbola is virtually linear, and rate is proportional to concentration of sucrose and may be determined by it. The hyperbola taken as a whole is the relation predicted between rate and substrate concentration for an enzyme obeying the Michaelis-Menten rule; i.e. one forming a simple enzyme-substrate compound whose rate of decay determines the rate of reaction. It is therefore possible that the respiration rate of the potatoes was determined by the relations between sucrose, or some substance varying with it directly, and a respiratory enzyme.

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[1] Widdowson, 1932.

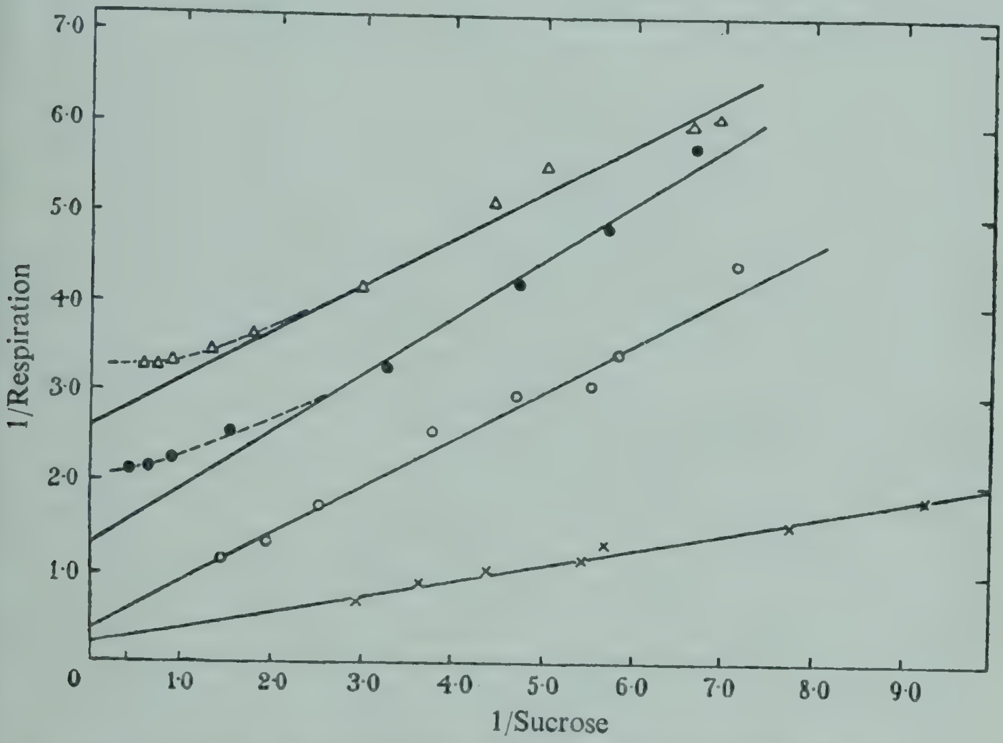

Fig. 40. Reciprocals of respiration rates and sugar concentrations; △, at −1° C.; ●, at +1° C.; ○, at +1° C.; ×, at 10° C. The first two series are increased by 2·0 and 1·0 respectively for convenience of graphing. After Barker, 1936.

INTERCONVERSION OF THE CARBOHYDRATES

The investigations just described make it clear that the labile carbohydrates are not separately consumed in respiration, but are interconvertible, the disappearance of one frequently coinciding with the accumulation of another. When an overall increase of carbon is ruled out, the labile polysaccharides are usually the first to decrease. They may therefore be called the ultimate respirable materials or reserves.

The commonest of them is starch; but it may be replaced by hemicelluloses in some seeds, inulin among some Compositae and monocotyledons, and fructosans in the Graminae.[1] Hemicelluloses include a wide range of natural products and many, like true cellulose itself, seem immune to respiratory breakdown, or are mobilized only in extreme starvation when irreversible degradations are far advanced.

Respirable carbon may also be obtained from glycosides which also vary greatly in the ease of their degradation *in vivo*. Examples of glycosides used in respiration are the prulaurasin of cherry-laurel leaves (p. 50) and salicin of willows.[2]

Many tissues contain no labile polysaccharides, and their role is then taken over by sucrose or, rarely, by the trisaccharide raffinose. Of these various substances, starch and sucrose are by so much the commonest that they may be regarded as the characteristic carbohydrate reserves, and the others as interesting occasional variants. Among actively respiring tissues, sucrose is sometimes present in the complete or virtual absence of starch, as in barley leaves and embryos or stored apples, allowing us to speak of a sucrose respiration. Starch, on the other hand, is always accompanied by sucrose if respiration is rapid, and is itself transformed to sucrose at least in part. The results just described do not indicate whether the carbon of starch passes wholly through sucrose before being respired, or whether some is able to follow a more direct route. In barley grains there is a spatial separation between starch hydrolysis leading to sucrose formation in the endosperm, and respiration of translocated sucrose in the embryo. As the embryonic respiration is estimated at nine-tenths of the whole, it seems probable that in these tissues the great bulk of the respired carbon must pass first through the form of sucrose. This may be exceptional, and there is no corresponding evidence for starch-containing barley leaves or for stored apples.

The disappearance of sucrose during mild starvation is associated with the formation of hexose; but the hexose that accumulates is not always the same. In barley leaves it is glucose, in Worcester Pearmain apples fructose, in Bramley's Seedlings, bananas,[3] and bean leaves a mixture of the two. Since sucrose is composed of a stable glucopyranose and a labile fructofuranose, it has been suggested that hydrolysis is followed by rapid consumption of the fructofuranose, and a slower removal of glucose. The suggestion was originally due to Mrs. Onslow (1931); and was based on the observation that the amount of hexose accumulating was approximately half the sucrose loss. To account for the fact that the hexose accumulating is frequently fructo- not glucopyranose it was necessary to suppose that glucose removal is not directly

[1] Archbold, 1940. [2] Weevers, 1948. [3] Barnell, 1943.

by respiration, but by way of conversion to fructose. There is direct evidence that glucose and fructose are interconvertible in plant cells, and that either may give rise to sucrose. Hartt (1937) fed the blades of sugar-cane leaves in the dark with 5 per cent. fructose and concentrations of glucose varying from 1 to 25 per cent. All the solutions led to large increases of sucrose formation in the blades. Similar results were obtained with albino as well as with green leaves, and there was no marked preference for either sugar. Sucrose synthesis by red clover, and wheat leaves supplied with either 10 per cent. glucose or 10 per cent. fructose has also been observed.[1] Barley leaves infiltrated with 10 per cent. glucose or 10 per cent. fructose also showed increased sucrose formation.[2] In the barley, clover, and wheat leaves, sucrose accumulated to about 6 per cent. of their dry weight and in the sugar cane up to about 9 per cent. The conversion is not limited to leaves, and has been shown to occur also in detached roots, stems, and entire stalks of sugar canes.[3] It also occurs in potato tubers.[4] The fact that sucrose may arise from either glucose or fructose suggests that the two hexoses must also be interconvertible. Direct investigation must be carried out with tissues previously freed from sucrose by starvation. Nurmia (1935) worked with young plants of wheat, oats, clover, and broad bean that had been kept in the dark for 24–48 hours. Cut stems or leaves were then fed with 10 per cent. glucose or 10 per cent. fructose solutions. There was a considerable synthesis of sucrose; but the results also showed that much of the supplied glucose had been converted to fructose and vice versa though, possibly, through sucrose; Hartt has shown that the same converse changes occur in etiolated sugar canes.

Phosphorylation of carbohydrates

Besides the sugars themselves, plant tissues contain a number of their phosphoric esters. A hexose monophosphate has been obtained from *Elodea*,[5] glucose and fructose monophosphates from sugar-beet leaves,[6] and a mixture of glucose and fructose monophosphates from garden-pea leaves.[7] Analytical data further suggest the occurrence of hexosemonophosphates in barley seedlings,[8] oat coleoptiles[9] and whole seedlings,[10] and potatoes.[11] Hexosediphosphate may be present in small amounts[9] or be undetectable.[8,11] Although it does not generally accumulate in intact tissues, it is generated in pea meal incubated with free phosphate,[1] and in spinach leaf *brei*,[2] and oat coleoptile extracts[3] incubated with glucose and ATP. It is now generally supposed that the interconversions of sugars in plant tissues are catalysed by the phosphate ion with the intermediary formation of such esters. Initial formation of the esters is probably similar in method to their formation in yeast. An enzyme, hexokinase, has been obtained from yeast,[4] which catalyses the transfer of phosphate ions from adenosine-triphosphate to glucose or fructose with the formation of glucose-6-phosphate and fructose-6-phosphate respectively. In yeast, supplies of adenosinetriphosphate are constantly regenerated, so continuous formation of the hexosephosphates may proceed. A similar origin has also been suggested for them in the higher plants,[5] and hexokinase activity has recently been stated to occur in mitochondrial preparations from avocados and the hypocotyls of mung bean seedlings.[6] Addition of glucose and catalytic amounts of adenosine triphosphate to mitochondrial preparations oxidizing α-ketoglutarate caused the disappearance from the medium of free phosphate which was presumed to be attached to the glucose. The esters formed have not yet been identified. The reverse change, hexosephosphate → hexose + H_3PO_4, is catalysed by phosphatases which release inorganic phosphate by hydrolysis. These enzymes are of wide distribution.[7] Glucose-1-phosphate is said to be much more resistant to their action than the 6-esters.[8]

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[1] Virtanen and Nordlund, 1934. See also Leonard, 1939, for maize, sorghum, and cotton leaves.

[2] McReady and Hassid, 1941.

[3] Hartt, 1937, 1940, 1943.

[4] Nelson and Auchincloss, 1933; Arreguin-Lozano and Bonner, 1949.

[5] Barrenscheen and Pany, 1930.

[6] Burkard and Neuberg, 1934.

[7] Hassid, 1938.

[8] Arney, 1939; Heard, 1945.

[9] Bonner, 1948.

[10] Albaum and Umbreit, 1943.

[11] Arreguin-Lozano and Bonner, 1949.

The hexosemonophosphates occurring in plant tissues have not yet been separately identified, and attempts resting on differential rates of hydrolysis are open to serious doubt.[9] The esters present probably include glucose-6-phosphate, fructose-6-phosphate, glucose-1-phosphate, and possibly fructose-1-phosphate.[10] These almost certainly exist in equilibrium with one another. Pea meal contains an isomerase that converts fructose-6-phosphate to a mixture with glucose-6-phosphate,[11] and a further enzyme which leads to the conversion of glucose-1-phosphate to the same mixture.[12]

In 1936 C. F. Cori and G. T. Cori showed that, in the presence of inorganic phosphate, the glycogen of muscle is converted by the enzyme phosphorylase to glucose-1-phosphate. A similar enzyme was also identified in other mammalian tissues and in yeast,[13] and the muscle enzyme was later purified and obtained in crystalline form.[1] Great interest attached to the discovery that the reaction was reversible and that a polysaccharide of high molecular weight, giving a blue colour with iodine, was obtainable from glucose-1-phosphate with simultaneous release of phosphate.[2] The existence of a similar enzyme in potatoes and peas has been shown by Hanes.[3] Crude extracts from potatoes contained relatively large amounts of phosphorylase, little amylase, and no enzyme capable of converting glucose-1-phosphate to other hexosemonophosphates. They therefore afforded particularly favourable material for the study of the plant phosphorylase, which did not need addition of adenylic acid as was necessary with the animal phosphorylases. Synthesis occurred providing small priming amounts of polysaccharides were present at the start. The polysaccharide formed *in vitro* closely resembled the amylose (straight chain) fraction of starch.[4] A similar enzyme has since been identified in a wide variety of plants.[5]

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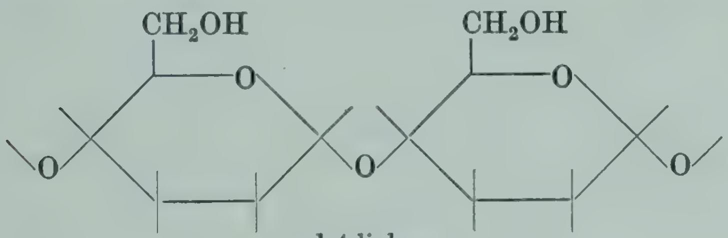
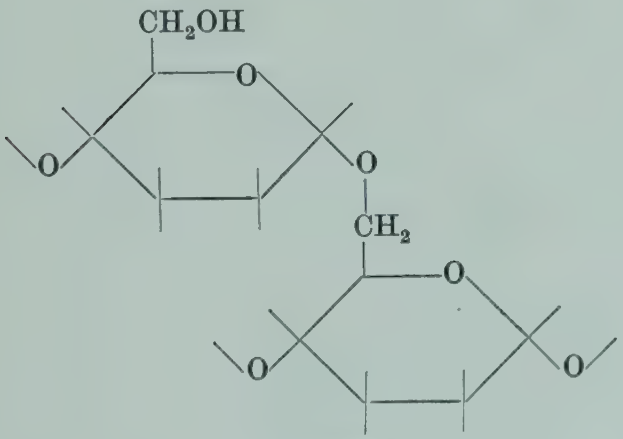
[1] Tanko, 1936.
[2] Bonner and Wildman, 1946.
[3] Bonner, 1948.
[4] Meyerhof, 1927, 1935*b*; Colowick and Kalckar, 1941.
[5] Hassid, 1943.
[6] Bonner and Millerd, 1952; Millerd, Bonner, and Biale, 1952.
[7] e.g. beans, potatoes, radishes, and wheat (Ignatieff and Wasteneys, 1936); sugar beet and potatoes (Pfankuch, 1936); iris, *Lamium* (Yin, 1945).
[8] Hassid, 1943.
[9] James, Heard, and James, 1944.
[10] Hassid, 1938.
[11] Tanko, 1936.
[12] Hanes, 1940*a*.
[13] Cori, Colowick, and Cori, 1938.

In addition to amylose, natural starches contain varying proportions of amylopectin. In this the chains built up by 1,4 linkages between glucose molecules are branched owing to the formation of occasional 1,6 linkages in addition. Phosphorylases catalyse the formation of



α 1,4 linkage



α 1,6 linkage

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[1] Green, Cori, and Cori, 1941.

[2] Cori, 1940; Kiessling, 1939.

[3] Hanes, 1940*a* and *b*.

[4] Astbury, Bell, and Hanes, 1940.

[5] Goddard and Meeuse, 1950, cite numerous references.

1,4 linkages to build up long straight chains containing more than 80 glucose radicles (amylose); but do not catalyse the formation of 1,6 linkages. This is supposed to result from the action of a second enzyme (Q-enzyme), which is also said to be extractable from potatoes.[1] A mixture of phosphorylase and Q-enzyme acting on glucose-1-phosphate *in vitro* produces amylopectin, and the Q-enzyme acting alone upon amylose also produces amylopectin, without the appearance of sugars or short-chain dextrins. The Q-enzyme thus appears to be responsible for introducing the branch linkages characteristic of the more complex starch component. Hydrolysis of the branch linkages is effected by a separate R-enzyme.

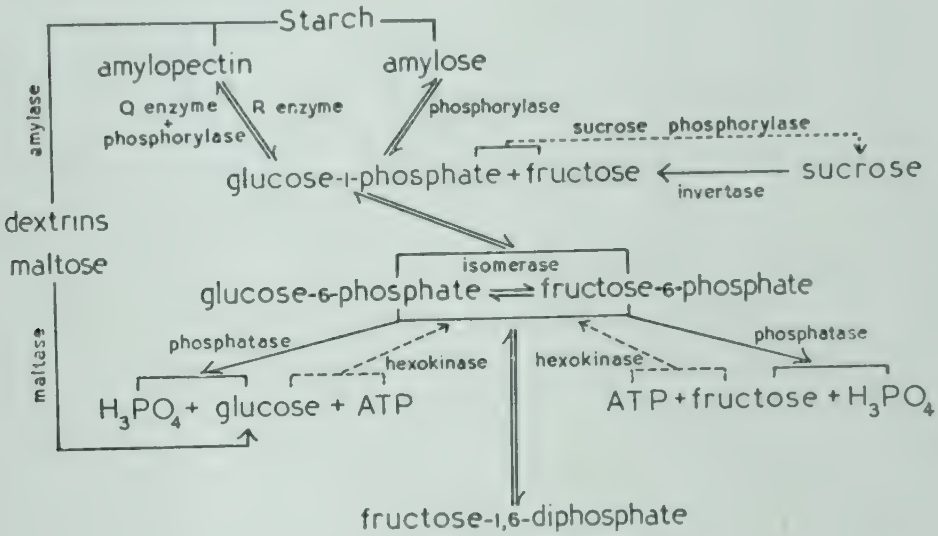
Starch
amylopectin
amylose
amylase
Q enzyme + phosphorylase
R enzyme
phosphorylase
sucrose phosphorylase
glucose-1-phosphate + fructose
invertase
sucrose
dextrins
maltose
isomerase
glucose-6-phosphate
fructose-6-phosphate
maltase
phosphatase
hexokinase
hexokinase
phosphatase
H_3PO_4 + glucose + ATP
ATP + fructose + H_3PO_4
fructose-1,6-diphosphate

FIG. 41. Scheme of carbohydrate relationships in plant tissues.

The interconversions of starch and hexoses can probably be represented by the above scheme (Fig. 41), in which the most doubtful step is the means of phosphorylation of the hexoses. The evidence for the hexokinase reaction in higher plants is largely analogy with other organisms. The actual fact of hexose phosphorylation is suggested by experiments with starch-free barley saps, in which addition of glucose, adenylic acid, and inorganic phosphate (or, alternatively, hexose-diphosphate alone) led to the production of pyruvic acid.[2]

The relation of sucrose to the other labile carbohydrates is more uncertain. Although sucrose accumulates rapidly when starch is disappearing (p. 104), its direct formation from starch appears to be ruled out by their respective molecular structures.[3] Recently a sucrose phosphorylase has been obtained from *Pseudomonas saccharophila*.[1] The enzyme catalysed the reversible reaction

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[1] Bourne and Peat, 1945; Bourne, Macey, and Peat, 1945.

[2] James, James, and Bunting, 1941.

[3] See also Meeuse, 1949.

$$\text{sucrose}+H_3PO_4 \rightleftharpoons \text{glucose-1-phosphate}+\text{fructose}.$$

Starting with glucose-1-phosphate and fructose, sucrose was obtained *in vitro*, and was shown to be identical with natural sucrose.[2] The fructose supplied was free fructopyranose, but it may be supposed that the phosphorylating reaction actually occurs with a small proportion of fructofuranose existing in equilibrium with the pyranose.[3] So far, no corresponding extraction of a sucrose phosphorylase from a higher plant has been claimed; though there is some evidence that phosphorylation is involved in sucrose ⇋ hexose conversions. Poisons known to inhibit phosphorylation have been shown to hinder sucrose formation.[4] Sucrose synthesis,[5] like interconversion of hexoses,[6] usually occurs only in the presence of oxygen, though it has been observed in sweet potatoes (*Ipomoea batatas*) kept under hydrogen.[7] The significance of these results is not clear; but it is possible that oxygen respiration is generally necessary for the regeneration of a phosphate donor in higher plants.

Whatever the precise mechanism may be, it seems clear enough that the sugars and labile polysaccharides are readily changed into one another in cells, and that the phosphate ion is an active agent. We thus have to do with a labile complex from which carbon may be removed by respiration at one or more points, and it will be shown later (p. 133) that hexosediphosphate is the most important point of withdrawal.

It is observable in the foregoing results that in phases of mild starvation starch and furanose anhydrides—fructosans, raffinose, sucrose, &c. —are consumed while pyranoses—glucose, fructose, and maltose—temporarily accumulate. This appears in the data for barley leaves (p. 103) and seedlings (p. 104), and for bulky fruits such as apples, pears, and bananas (p. 106).[8] It may also occur in rye stalks, although the situation is somewhat complicated by simultaneous translocation into the ear.[9]

The ready utilization of sucrose was ascribed by Onslow (cf. p. 110) to a preferential consumption of its unstable fructofuranose. Such an explanation would not account for the equally ready utilization of starch, known to be a pyranose anhydride. Once it is established that respiration proceeds by way of phosphorylation, the special respiratory position of starch and the furanosides is not difficult to explain. Being already condensed, they contain ester linkages not present in the simple hexoses, and, as a result, the replacement of hexose-hexose linkages by hexose-phosphate linkages may proceed with little change of free energy, whereas the creation of the hexose-phosphate linkage from free hexose and free phosphate is markedly endergonic. A similar situation has been observed in muscle where glycogen is more readily utilized than glucose. The energy relations of the system have been studied, and there is no reason to suppose that they differ essentially from those of starch transformations in the cells of plants. The reactions concerned are shown in Fig. 42.

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[1] Doudoroff, 1943; Doudoroff, Kaplan, and Hassid, 1943.

[2] Hassid, Doudoroff, and Barker, 1944.

[3] Street, 1950.

[4] Hartt, 1943, 1944; Kriukova, 1940 (iodoacetate).

[5] Boysen-Jensen, 1912 (barley and peas); Leonard, 1939 (sorghum).

[6] Hartt, 1944 (sugar cane).

[7] Hasselbring, 1918.

[8] See also Barnell, 1943, for banana pulp.

[9] Belval, 1924.

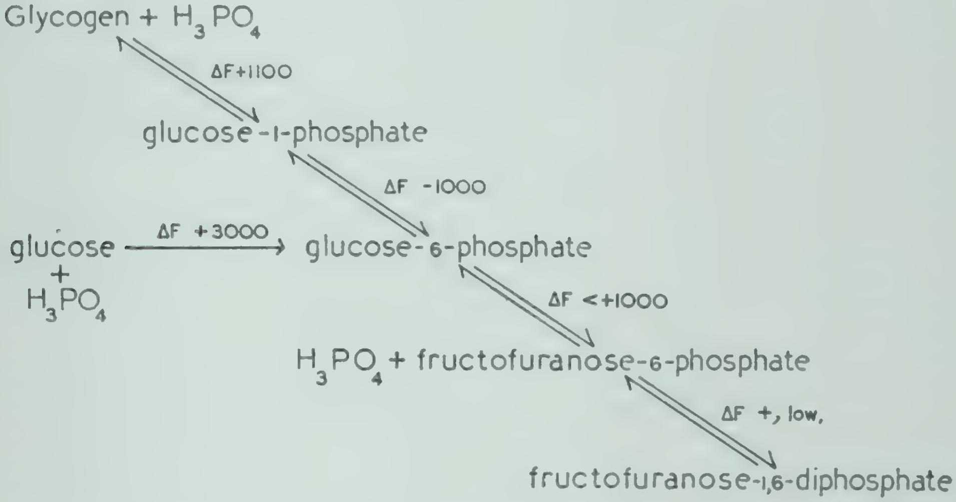

FIG. 42. Relations of phosphoric esters and glycogen.

All these phosphate esters fall into Lipmann's (1941) energy-poor category, with a linkage-energy approximately equal to that of the glucose-glucose link in glycogen, i.e. about 3,000 cal. In passing from glycogen+H_3PO_4 to glucose-6-phosphate there is virtually no change of free energy. In passing from glucose +H_3PO_4 to glucose-6-phosphate, there is, on the other hand, an increase of free energy of about 3,000 cal. This is provided from the carrier adenosine triphosphate (ATP), rich in transferable energy, which in its turn must be generated by respiratory or glycolytic oxidations at a cost of about 11,000 cal. Neither the phosphorylation of glycogen[1] nor apparently the phosphorylation of starch needs any similar energy boost; both are independent of ATP formation.

The furanosides would appear to be in a slightly more favourable position still, since furanose linkages are more labile than pyranose.

[1] Lipmann, 1941; Kiessling, 1939.

The furanosides are characterized by hydrolysis rates about 100-fold faster than those of pyranosides;[1] the hydrolysis of fructofuranose-6-phosphate is about 7 times faster than that of glucopyranose-6-phosphate.[2] In the equilibrium between the two last, catalysed by oxoisomerase, there is 30 per cent. of the furanose ester and 70 per cent. of the pyranose ester, corresponding with a $\Delta F < +1{,}000$ cal. in passing from the pyranose to the furanose ester. The increase is quite small; nevertheless, it is noticeable that addition of a second phosphate radicle to give hexosediphosphate only occurs after the glucopyranose monophosphate has been converted to its fructofuranose isomer. It is, in fact, possible that the latter can be phosphorylated without the utilization of an energy-rich carrier.[3] Any special contribution of the furanose structure to facilitating respiration is likely to lie in providing more readily phosphorylated hexose rather than in providing a structure which is, in the free form, itself more easily decomposed.

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[1] Armstrong and Armstrong, 1934.

[2] Umbreit, Burris, and Stauffer, 1949.

[3] Cf. Lipmann, 1941.

VI

GLYCOLYSIS

De Saussure (1804) and other nineteenth-century investigators observed that plant tissues, enclosed in atmospheres from which the oxygen had been removed, continued to give off carbon dioxide.[1] Bérard (1821) found that ripe fruits in this predicament not only continued to give off carbon dioxide, but at the same time lost so much of their sugar, 'as by a sort of fermentation', that they seemed more acid, although their acids had not actually increased. Later Lechartier and Bellamy[2] demonstrated the production of alcohol by apples in amounts almost equal to the CO_2 emitted. To answer the objection that the alcohol might have been formed by epiphytic yeasts, they cut their material for distillation from the centre of the fruit. It was Pasteur, in pursuit of his thesis that fermentation is life without oxygen, who first gave significance to these observations. He wrote: 'To sum up, fermentation is a very general phenomenon. It is life without air, it is life without free gaseous oxygen, or, still more generally, it is the result of a chemical process accomplished by means of a fermentable substance capable of producing heat by its decomposition.' He went on to show that several plant tissues—a bunch of grapes, a melon, oranges, plums, and rhubarb leaves—kept under an atmosphere of carbon dioxide, continued to emit CO_2 and accumulated alcohol. He was unable to discover any yeast cells within the tissues, and the chemist Dumas, visiting his laboratory also made the examination and expressed himself satisfied that there were none. Pfeffer[3] at a later date was more sceptical. From a sample of Orleans plums kept for 8 days under carbon dioxide, Pasteur distilled 6·5 gm. alcohol, more than 1 per cent. of their total weight. In view of an assumption so frequently made by plant physiologists since his time, it is interesting to note that these observations on fruits inspired him to comment: 'It must be understood that the equation of a fermentation is essentially variable with the conditions under which it occurs.' He thought it an 'abuse of language' to speak of the alcohol production in fruits as 'alcoholic fermentation', since the products had not been shown to be formed in the same proportions as by yeast. At the present time the Gay-Lussac equation for alcoholic fermentation

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

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[1] The following are cited by Kostychev, 1927: Rollo, 1798; Dumont, 1819; Bérard, 1821; Döbereiner, 1822; Böhm, 1873.

[2] Lechartier and Bellamy, 1869, 1872.

[3] *Physiology of Plants.* English trans., vol. i, p. 539.

has still to be verified quantitatively for the anaerobic respiration of any higher plant.

Observations of anaerobic alcohol formation by plant tissues continued to be made at frequent intervals. De Luca (1879) enclosed various leaves, flowers, and fruits in hydrogen, carbon dioxide, or *in vacuo*. He described his researches as 'tending to show the production of alcohol'; the criterion of alcohol formation being the distillation of a volatile liquid which, on warming, gave off an inflammable vapour. Matruchot and Molliard, doubting the absence of micro-organisms from the experimental material of earlier workers, obtained and demonstrated conditions of strict asepsis. Pieces of beetroot were shown to give off massive amounts of CO_2; but the only identification of alcohol seems to have been by smell. Palladin and Kostychev (1906) reported anaerobic alcohol formation in peas, castor-oil seeds, wheat grains, lupin seeds and seedlings. They depended on identifying the smell of benzoic ethyl ester after suitable treatment of the distillate, together with a positive iodoform reaction as previously used by Muntz.[1] Since this reaction is also given by volatile aldehydes and ketones, they showed the absence of these by means of Schiff's fuchsin reaction. With the distillate from wheat grains this reaction was positive, and they confirmed the presence of traces of acetone by several other test reactions.

So recently as 1947 it has been remarked that 'little experimental proof exists that the product of fermentation in plants is actually ethyl alcohol'. The writer[2] attempted the identification by comparing the refractive index with the dichromate-reducing power of suitably prepared distillates. Distillates from barley seedlings gave agreement within 3–4 per cent.; those from rice seedlings only within about 10 per cent.; but they boiled at 78–79° C. (b.p. ethyl alcohol = 78° C.). Probably the most satisfactory identification is by formation of dinitrobenzoic ethyl ester in benzene solutions of the distillate. This yields a crystalline solid with sharply defined melting-point; it has been achieved with barley grains, apples, carrots, and spadix of *Arum maculatum*.[3] The yield from 950 gm. intact apples kept for 50 hours under nitrogen was 0·75 gm. melting at 93° C. (m.p. ethyldinitrobenzoate = 94° C.); from 575 gm. *Arum* spadix, 0·27 gm. was obtained after 24 hours (m.p. = 94° C.). The surface of the tissues was unbroken, and they were apparently unchanged in condition after their short anaerobiosis. There was no indication of bacterial contamination by micro-organisms.

Alcohol formation also appears to occur to a limited extent in air. Palladin and Kostychev found determinable amounts in the tips of fresh broad-bean stems, and in germinating wheat and peas; there were

[1] Muntz, 1876, 1878.

[2] Phillips, 1947.

[3] B. H. Thewlis, 1952.

also traces in seedlings of *Lupinus luteus*.[1] Similar accumulations are found in barley; but formation in germinating grains is ambiguous because anaerobic conditions are inevitably set up within them for a short time (cf. p. 92), and the alcohol once formed is not destroyed when aerobic conditions return. Phillips (1947) found that alcohol continued to increase in germinating barley for about 48 hours when adequate aeration had apparently been restored. Aerobic alcohol formation has also been observed in fleshy fruits, and is usually accompanied by small accumulations of acetaldehyde, as in tomatoes and also in their leaves.[2] In apples the amount increases very much as the fruits age,[3] reaching a maximum with other volatiles at the climacteric, or if they are injured.[4] Potatoes, germinating peas,[2] and apical meristems[5] have also been shown to accumulate alcohol in air.

ALCOHOL/CARBON DIOXIDE RATIO

Much attention has been given to the experimental determination of this ratio. In the alcoholic fermentation equation, the molecular ratio $C_2H_5OH/CO_2 = 1{\cdot}00$, and in yeast this value may be realized to a close approximation. The estimation of carbon dioxide production with a sufficient degree of accuracy during a period of anaerobic respiration presents little difficulty; but the estimation of the alcohol is less easy. It requires a quantitative recovery of the alcohol by fractionation and distillation followed by a determination of the alcohol in the aqueous distillate. Methods used have been physical, e.g. determination of the specific gravity,[6] depression of the freezing-point,[7] or the refractive index[8] of the water-alcohol mixture; or chemical, usually the quantitative oxidation of the alcohol by bichromate in the presence of sulphuric acid.[9] Alcohol added to crushed tissues can be determined by the last method with an error of 1–2 per cent. A small amount of acetaldehyde is usually present, and most investigators include this with the alcohol when calculating the alcohol/CO_2 ratio, on the grounds that it is an immediate precursor in alcohol formation. Usually the amount of acetaldehyde accumulating is, as might well be supposed, very small; in barley it appears to be about 1 per cent. of the amount of alcohol.[8] In normal apples it is stated to be negligible,[10] but in apples stored under carbon dioxide it may equal more than 50 per cent. of the alcohol.[1] The bichromate oxidation method will not distinguish between acetaldehyde and alcohol, so that a correction must be made by removal or separate estimation of the acetaldehyde, if appreciable.

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[1] They also cite similar results by Mazé, 1899, 1902, and Polovzov, 1901.

[2] Gustafson, 1934.

[3] Fidler, 1933*a*.

[4] Thomas, 1931.

[5] Ruhland and Ramshorn, 1938.

[6] e.g. Palladin and Kostychev, 1906.

[7] e.g. Palladin and Lvoff, 1913.

[8] Phillips, 1947.

[9] Kostychev, 1913; Phillips, 1947; Barron, Link, Klein, and Michel, 1950; see Fidler, 1934, for critical investigation of the methods with apples.

[10] Less than 1/75 alcohol concentration in Bramley's Seedling and Newton Wonder, Fidler, 1933*a* and *b*.

Lechartier and Bellamy (p. 118) thought the alcohol they detected in apples to be formed in amounts almost equal to the carbon dioxide emitted. Godlewski and Polsenius (1901) were the first to submit quantitative data, obtaining values of the ratio for germinating peas varying from 1·02 to 0·73.[2] On the basis of these results they proposed that the anaerobic respiration of higher plants was an alcoholic fermentation. Some later investigations have also given ratios approximating closely to unity; but it is quite clear that the ratio sometimes falls much lower than can reasonably be ascribed to experimental error (Table 25); in short, there is often more carbon dioxide produced than can be accounted for by an unmodified fermentation of carbohydrates to alcohol.

Extracted enzymes in the press-juice of peas, potatoes, and beet were found to give ratios very near theoretical,[3] and a satisfactory explanation cannot always be given of the discrepancy with intact tissues. Additional carbon dioxide might be given off if an increase of acidity released previously bound CO_2. This may happen to some extent in barley seedlings,[4] potatoes, and *Chlorella* suspensions[5] as a temporary effect, not a long-continued one. It has also been suggested that anaerobic carbon dioxide may be liberated by the breakdown of other than carbohydrate reserves. Nabokich pointed out that castor-oil seedlings gave lower values of the ratio than peas, and subsequent results with fat-storing seeds have also been low (Table 25). This explanation, even if verified, would only cover a small proportion of the results; breakdown of protein does not lead to CO_2-production under nitrogen (see p. 60). Departure of the ratio from unity does not necessarily imply any drastic deviation *ab initio* from an alcoholic fermentation. Apples with a negligible accumulation of acetaldehyde may show a ratio as high as 0·97; but in a carbon dioxide atmosphere, with about one-third of the alcohol remaining as acetaldehyde,[1] the strict C_2H_5OH/CO_2 ratio might be expected to fall to 0·65. It is not certain that, in the event of such a failure of the last stage, the missing alcohol would accumulate as acetaldehyde, which would, indeed, be more likely to condense or undergo some other fixation. Palladin[6]

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[1] Thomas, 1925.

[2] Many authors give the ratio as 100 gm. CO_2 : gm. alcohol. The equation then requires 100:104·5. All results are given in the text as the molecular ratio C_2H_5OH/CO_2.

[3] Stoklasa and Czerny, 1903; Bodnar, 1916.

[4] Phillips, 1947.

[5] Boswell and Whiting, 1940.

[6] Palladin, 1912*a*.

TABLE 25

Molecular ratio of ethyl alcohol/carbon dioxide in anaerobic respiration

		C_2H_5OH/CO_2	
LEAVES .	*Acer platanoides*	0·55	Kostychev, 1913
	Syringa vulgaris	0·54	,, ,,
	Prunus padus	0·49	,, ,,
	Tropaeolum majus	0·43–0·16	Boysen-Jensen, 1923
ROOTS . .	Carrots	0·98	Kostychev, 1913
	,,	0·89–0·69	Boysen-Jensen, 1923
	,,	1·08–0·96	Wetzel, 1933
	Brassica rapa	0·47	Kostychev, 1913
TUBERS .	Potato	0·07–0·00	Boysen-Jensen, 1923
	,, dormant	0·07	Kostychev, 1913
	,, sprouting	0·00	,, ,,
	,, sweetened	0·17	,, ,,
	,, cut	0·34–0·27	,, ,,
	,, slices	0·91[1]	Barron *et al.*, 1950
FRUITS .	Apples, 'Sinap'	0·77	Kostychev, 1913
	,, 'Anton'	0·40	,, ,,
	,, numerous varieties	0·97–0·47	Fidler, 1933*b*
	Oranges	0·67	Kostychev, 1913
	Blue grapes	0·91–0·71	Boysen-Jensen, 1923
	Green grapes	0·82–0·71	,, ,,
SEEDLINGS .	*Lepidium sativum*	0·55	Kostychev, 1913
	Sinapis sp.	0·48–0·31	Boysen-Jensen, 1923
	Peas	1·02–0·73	Godlewski and Polsenius, 1901
	,,	1·03–0·96	Nabokich, 1903*a* and *b*
	,,	0·70	Palladin and Kostychev, 1906
	,, cotyledons	0·78–0·62	Boysen-Jensen, 1923
	Beans	0·48–0·25	Palladin and Kostychev, 1907
	Lupinus luteus	0·87–0·60	Palladin and Kostychev, 1906
	Ricinus communis	0·57	Palladin and Kostychev, 1906
	,, ,,	0·83–0·49	Nabokich, 1903*b*
	Barley	0·87[1,2]	Phillips, 1947
	Rice, 1st leaf not out	1·06–0·98	,, ,,
	,, ,, emerging	0·76	,, ,,
	,, ,, well out	0·54	,, ,,

[1] Calculated from author's data.
[2] After an initial period of about 6 hours.

observed that tissues with abundant chromogens (p. 179) were particularly liable to show low alcohol/CO_2 ratios, and that, after disruption by freezing, they might form no alcohol at all. He supposed that transferable hydrogen in these tissues, instead of reducing fermentation intermediates, was diverted to reduction of the chromogens. No systematic search for products of anaerobic respiration other than alcohol has yet been undertaken, and so it is impossible to say how widely it deviates from a pure alcoholic fermentation.

The observation of $C_2H_5OH/CO_2 = 1$ is consistent with the sole production of ethyl alcohol, but does not prove it. As will be seen later (p. 128), the first release of carbon dioxide occurs when pyruvic acid is decarboxylated to acetaldehyde, i.e. at a fairly late stage of the sequence. Any deviation of the reaction path at an earlier stage would not necessarily be reflected in the ratio, since it might be that neither CO_2 nor alcohol was produced. Thus the formation of phosphoglycerol by reduction of triosephosphate with possible accumulation of free glycerol, as in the bisulphite-inhibited fermentation of yeast, or the formation of lactic acid by the reduction of pyruvic acid, might account for a large part of the sugar breakdown without disturbing the alcohol/CO_2 ratio at all. In view of the massive formation of lactic acid in muscle glycolysis, it is interesting that its formation has also been observed in some higher plant tissues.[1] The most extensive investigation has been by Schneider, who isolated the lactic acid as zinc lactate, and estimated it gasometrically as carbon monoxide after treatment with concentrated sulphuric acid. Simultaneous determinations of malic and citric acids were also carried out. He found lactic acid so generally present in a wide range of plant tissues that he considered it must be 'a normal product of plant metabolism'. It was particularly conspicuous in maize embryos, where it exceeded the amounts of both citric and malic acids. Of the total acidity 50 per cent. was due to lactic acid, 13·3 per cent. to citric, and 8·9 per cent. to malic. Similar results were obtained with broad beans; in lettuce leaves, the acid distribution was 17·6 per cent. lactic, 5 per cent. citric, and 57·6 per cent. malic. During the germination of seeds there was a sudden increase to a maximum, particularly noticeable in maize, which appeared to correspond with the period of temporary anaerobiosis experienced by many seeds during early germination (p. 92). Schneider was not, however, able to show an increase of lactate formation when the grains were placed under nitrogen as was shown by Phillips with barley.

THE RATIO HEXOSE/ALCOHOL+CO_2

The identity of an anaerobic respiration with alcoholic fermentation could only be shown by equating the sugar loss with the production of ethyl alcohol and carbon dioxide. This has not been done for any of the higher plants. Nabokich (1903*b*) did, however, perform a remarkable series of experiments with pea seedlings in which he estimated the loss of dry weight as well as the production of CO_2 and alcohol during anaerobiosis. Calculating on the assumption that starch is the only material consumed, he obtained the following series of results.

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[1] e.g. by Phillips, 1947 (barley seedlings); Barron, Link, Klein, and Michel, 1950 (potato slices); Barker and el Saifi, 1952 (intact potatoes).

TABLE 26

Loss and gain of material during anaerobic respiration by pea seedlings

Data of Nabokich, 1903*b*

Duration of experiment (days)	*Loss of*		*Gain in*			*Difference (%)*
	Dry wt. (gm.)	*Hexose equivalent (gm.)*	CO_2 *(gm.)*	*Alcohol (gm.)*	*Total (gm.)*	
14	4·268	4·715	2·331	2·431	4·762	+1·0
14	4·915	5·407	2·597	2·706	5·303	−1·9
14	4·769	5·242	2·484	2·677	5·161	−1·6
16	3·916	4·198	1·935	2·055	3·390	−4·9
16	3·732	4·105	1·993	2·076	4·069	−0·9
15	3·282	3·610	1·756	1·771	3·527	−2·3
24	4·055	4·461	2·164	2·233	4·397	−1·4

Agreement in these figures is remarkably close with a mean deficiency of only 2·0 per cent., which could be accounted for by a small increase in non-volatile acids as with yeast. As the alcohol/CO_2 ratio also varied only from 1·03 to 0·96 it must be considered that a good case was made for considering the anaerobic respiration of these peas to correspond with alcoholic fermentation.

Actual determination of sugar loss has recently been carried out by Phillips Nance using detached barley roots kept 4 hours under nitrogen. The mean loss of sugar (hexose+sucrose) by 10 gm. root samples was equivalent to 0·009 m.M. of hexose.[1] The simultaneous production of alcohol+acetaldehyde was 0·017 m.M. This agrees well with the requirement of 2 molecules of alcohol from each molecule of sugar; but in view of the sampling errors needs confirmation. The carbon dioxide produced was, however, greatly in excess, varying from 0·10 to 0·27 m.M. Although the differences between samples were so great, it seems clear that a large part of the carbon dioxide must have come from non-carbohydrate sources.

The results of a large-scale investigation on apples have recently been published by Fidler.[2] Owing to inevitably high sampling errors, the data obtained were rather complex, but amenable to statistical analysis. It was found that, during the prolonged storage periods, malic acid steadily disappeared from the slowly ripening apples, and that the rate of its consumption was unaltered by the removal of oxygen. It would therefore seem reasonable to attribute any extra anaerobic CO_2-

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[1] Individual determinations varied from 0·003 m.M. to 0·014 m.M.

[2] Fidler, 1948, 1951.

production, over the requirements of the fermentation equation, to the loss of malic acid. Working on this basis it was found that each molecule of extra CO_2 would be equivalent to one carbon atom lost as acid; in other words that the total anaerobic CO_2-output was equivalent to the loss of labile carbohydrate+malic acid, presuming the acid to be wholly converted to CO_2. This implies the complete oxidation of the acid with the simultaneous formation of a considerable quantity of reduced compounds in the fruit, which have not been discovered. It is not clear from the published accounts of this work that the fruit remained entirely sterile during the long periods, up to 70 days, under nitrogen. Most of the experiments were, however, performed at 12° C. at which temperature contaminations might not develop rapidly.

The effect of the various results described above is to suggest that an alcoholic fermentation is the predominant reaction in the anaerobic respiration of higher plants. There is equally good evidence that this is frequently accompanied by collateral or side reactions that lead to a considerable excess of carbon dioxide over alcohol production. To attempt to assess anaerobic glycolysis rates from CO_2-production alone may be liable to large errors.[1]

The extraction of zymase from higher plant tissues was first claimed by Stoklasa and Czerny in 1903; but the claim led to considerable controversy and some confusion. Others were unable to repeat their results and criticized their methods.[2] Some extracts gave vigorous glycolysis without any alcohol formation,[3] and plants killed by freezing emitted carbon dioxide; but produced little or no alcohol.[2] In the light of after knowledge it appears that much of the misunderstanding arose from a failure to appreciate the complexity of the system being investigated. The earlier workers seem to have thought of zymase as a single enzyme instead of the complicated mixture of enzymes and accessory factors that it is now known to be. The wonder is not that some tissues failed to convert sugar to alcohol after disorganization, but that others did. A long controversy on the nature of anaerobic respiration continued during the early years of the century until it was extinguished in 1914. Several significant facts were brought to light: that it was possible to catalyse the conversion of sugar to alcohol by at least some post-mortem plant systems; that addition of inorganic phosphates might increase their CO_2-production; and that partly fermented sugar solutions or boiled yeast extracts might do the same.[4] Finally Bodnar (1916), reinvestigating the extraction of zymase with detailed precautions to avoid microbial contaminations, provided adequate confirmation of

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[1] Cf. Boswell and Whiting, 1940.

[2] See Palladin and Kostychev, 1906.

[3] Hahn, 1900.

[4] For a full summary of the work up to 1914 see Onslow, 1931, and Kostychev, 1927.

Stoklasa's original claim. Juice was pressed from beet and potatoes at 300–400 atmospheres, passed through linen, and precipitated by alcohol. It was incubated with 15 per cent. glucose at 37° C. Carbon dioxide and alcohol were measured and found to be formed in almost theoretical proportions.

TABLE 27

Zymase activity in press-juice of plant tissues

C_2H_5OH/CO_2 ratios

Beet		*Potatoes*		*Peas*
Stoklasa	*Bodnar*	*Stoklasa*	*Bodnar*	*Stoklasa*
0·936	0·965	1·311	1·008	0·998
1·416	0·968	0·852	0·998	1·056
1·034	1·135		0·881	1·055
1·048	1·011		0·994	
1·148				
1·181				
0·994				
Mean 1·108	1·019	1·082	0·970	1·036

Significance attaches specially to the high yield of alcohol with press-juice of potatoes, because the intact tubers formed no alcohol at all (Table 25). Intermediate degrees of laceration produced moderate proportions of alcohol, suggesting that the greater the degree of disorganization the more the anaerobic respiration swung towards alcohol formation. After about fourteen days under nitrogen, even intact tubers begin to form large amounts of alcohol; before that they form lactic acid and other alcohol-soluble solids.[1] A small increase of acids has been observed in anaerobic pea[2] and barley[3] seedlings, and occurs even in yeast; it may be that what are side reactions in these organisms become the principal reaction path in potatoes.

THE STAGES OF GLYCOLYSIS—THE YEAST-MUSCLE SCHEMA

Ever since the original suggestion by Pasteur, the study of anaerobic respiration has been dominated by the idea of its similarity to alcoholic fermentation. Further development had to await that of a comprehensive theory for yeast, which offers many advantages to the experimentalist denied by higher plants. The unravelling of this complex metabolic mechanism has been one of the major achievements of modern biochemistry, and has been the work of numerous investigators. At the same time, the study of glycolysis in muscle has made clear the essential similarities between the two processes in spite of superficial differences. So closely allied are they in their principal reactions that it is possible to represent them by a common schema with simple variations. The study of glycolysis in the higher plants has not yet proceeded so far; but it seems reasonably well established that it, too, follows the same path in essentials. At least in the early stages of glycolysis, we seem to recognize a hard core of metabolism that exists almost unaltered in the most diverse organisms, which may have little else to show in common, which may differ markedly in the products of their metabolism, and even essentially in their methods of assimilation. We may therefore regard the yeast-muscle schema as a sort of base from which to attack the problems of plant glycolysis. A discussion of its development falls outside our scope, and is unnecessary by reason of numerous presentations recently available.[1] The stages at present accepted are set out in Fig. 43. The formation of fructofuranose-1,6-diphosphate from hexose or storage polysaccharides has already been considered (p. 114) in relation to other sugar changes. The following features of the succeeding reactions are important.

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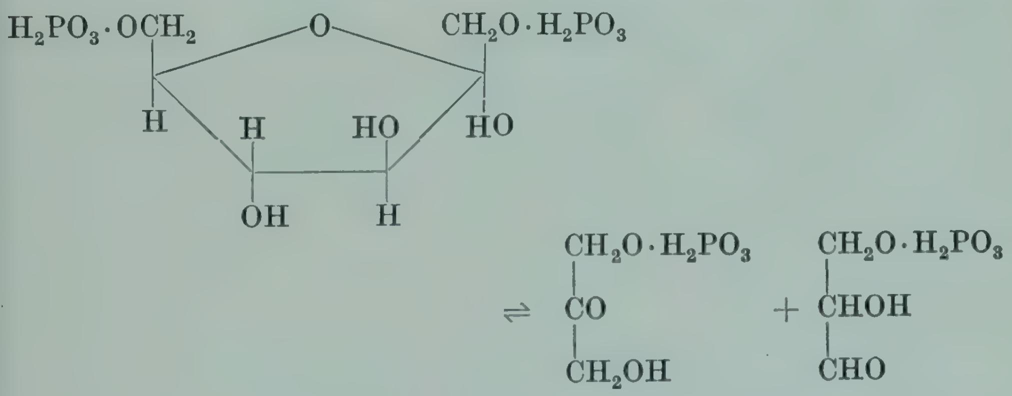
[1] Barker and el Saifi, 1952.

[2] Nabokich, 1903*b*.

[3] Phillips, 1947.

1. *Splitting of hexosediphosphate* is catalysed by the enzyme zymohexase (aldolase) to yield 1 molecule dihydroxyacetone phosphate and 1 molecule 3-phosphoglyceraldehyde. The enzyme phosphotriose isomerase catalyses the interconversion of the two triosephosphates. The initial reaction is also reversible and, *in vitro*, an equilibrium mixture of the three reactants is rapidly achieved, whether hexosediphosphate or a triosephosphate is the starting-point.[2]



2 and 3. *Phosphorylation and oxidation of 3-phosphoglyceraldehyde.* The subsequent intermediates of glycolysis derive from the phosphoglyceraldehyde, both hexosediphosphate and dihydroxyacetone phos-

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[1] As, for example, Mann and Lutwak-Mann, 1944; Baldwin, 1948; Stevenson, 1949; Bell, 1952; Sumner and Somers, 1947; Barron, 1943.

[2] Meyerhof and Lohmann, 1934; Meyerhof, Lohmann, and Schuster, 1936.

phate being continuously converted into it as removal proceeds. It was shown in 1939[1] that the product of oxidation was 1,3-diphosphoglyceric acid, and that, when a highly purified crystalline enzyme (triosephosphate dehydrogenase) was used, it was necessary to add inorganic phosphate before the reaction would proceed. To account for these results it may be supposed that 1,3-diphosphoglyceraldehyde is first formed (reaction 2).

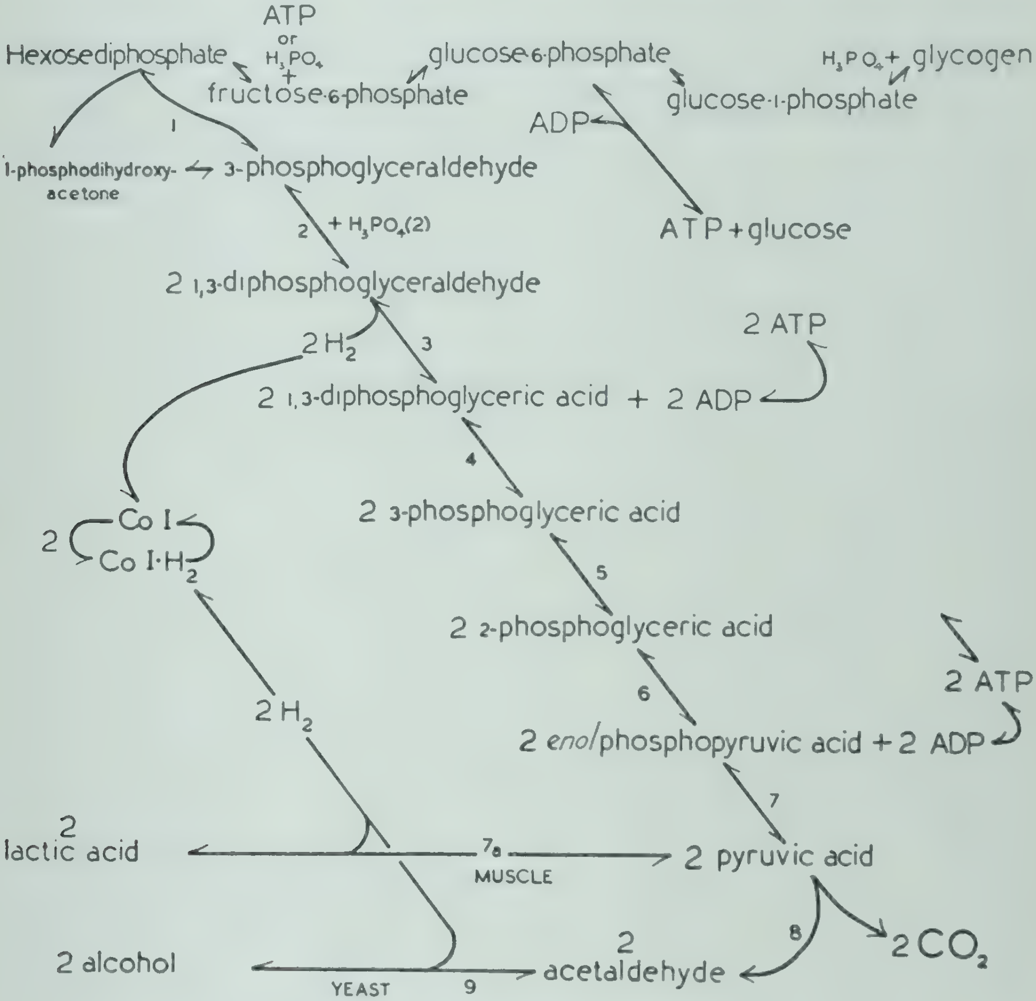
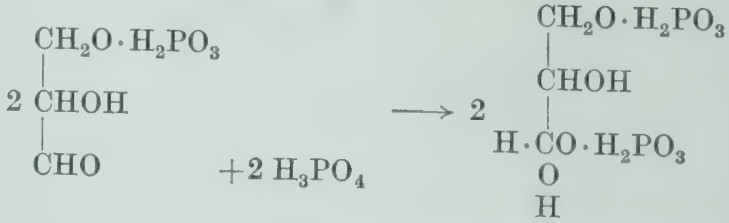

ATP
or
H₃PO₄
+
Hexosediphosphate
fructose-6-phosphate
glucose-6-phosphate
H₃PO₄ + glycogen
glucose-1-phosphate
ADP
1
1-phosphodihydroxy-acetone
3-phosphoglyceraldehyde
2
+ H₃PO₄(2)
ATP + glucose
2 1,3-diphosphoglyceraldehyde
2 H₂
3
2 ATP
2 1,3-diphosphoglyceric acid + 2 ADP
4
2 Co I
Co I·H₂
2 3-phosphoglyceric acid
5
2 2-phosphoglyceric acid
2 H₂
6
2 ATP
2 enolphosphopyruvic acid + 2 ADP
7
2 lactic acid
7a
MUSCLE
2 pyruvic acid
8
2 CO₂
2 alcohol
YEAST
9
2 acetaldehyde

FIG. 43. Yeast-muscle schema of glycolysis.

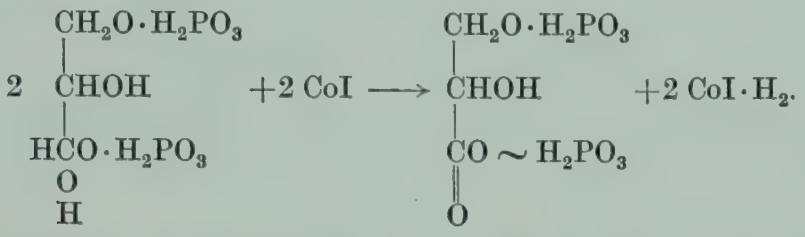


$$2\begin{array}{l}CH_2O\cdot H_2PO_3\\ |\\ CHOH\\ |\\ CHO\end{array} + 2H_3PO_4 \longrightarrow 2\begin{array}{l}CH_2O\cdot H_2PO_3\\ |\\ CHOH\\ |\\ H\cdot\underset{\underset{\displaystyle H}{\displaystyle O}}{C}O\cdot H_2PO_3\end{array}$$

---

[1] Warburg and Christian, 1939; Negelein and Brömel, 1939.

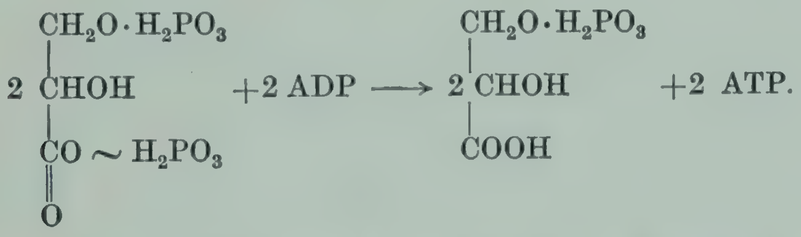
Inorganic phosphate is used, ATP (adenosine triphosphate) not being needed, and it is doubtful whether any enzyme is needed either. The aldehyde is then dehydrogenated by triosephosphate dehydrogenase (reaction 3) requiring Coenzyme I as the hydrogen acceptor.



$$2\ \begin{array}{l} CH_2O\cdot H_2PO_3 \\ | \\ CHOH \\ | \\ HCO\cdot H_2PO_3 \\ O \\ H \end{array} + 2\ CoI \longrightarrow \begin{array}{l} CH_2O\cdot H_2PO_3 \\ | \\ CHOH \\ | \\ CO \sim H_2PO_3 \\ \| \\ O \end{array} + 2\ CoI\cdot H_2.$$

Triosephosphate dehydrogenase is inhibited by M/1,000 iodoacetate[1] which reacts with its thiol (—SH) groups.[2] Application of this poison therefore blocks the further course of fermentation, and triosephosphates tend to accumulate. When this happens, part of the 3-phosphoglyceraldehyde acts as hydrogen acceptor from the reduced Coenzyme I ($CoI\cdot H_2$) and phosphoglycerol appears. In unpoisoned fermentation, more efficient H-acceptors are formed and no phosphoglycerol. Dialysis, leading to removal of Coenzyme I, also inhibits. When oxidation by removal of 2H from the C-atom 1 occurs, the compound is activated to the energy-rich condition.[3]

4. *Dephosphorylation of 1,3-diphosphoglyceric acid.* In muscle extracts, the oxidation of triosephosphate by transfer of 2H via Coenzyme I is coupled with synthesis of ATP.[4] For each molecule oxidized one phosphate radicle is esterified. The energy-rich phosphate in 1,3-diphosphoglyceric acid is transferred to adenosine diphosphate to form the energy-rich ATP.



$$2\ \begin{array}{l} CH_2O\cdot H_2PO_3 \\ | \\ CHOH \\ | \\ CO \sim H_2PO_3 \\ \| \\ O \end{array} + 2\ ADP \longrightarrow 2\ \begin{array}{l} CH_2O\cdot H_2PO_3 \\ | \\ CHOH \\ | \\ COOH \end{array} + 2\ ATP.$$

In this important reaction, part of the energy of the oxidation is stored in the ATP in a form that is capable of further transfers instead of being lost to the organism (cf. p. 243). The enzyme concerned, if any, is unidentified.

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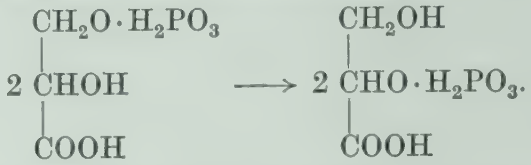
[1] M. Dixon, 1937; Adler, Euler, and Günther, 1938; Meyerhof and Kiessling, 1935.

[2] Rapkine, 1933.

[3] ~ indicates that much energy may be transferred with the phosphate, not that it is all in this linkage.

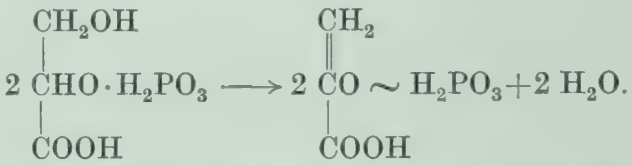
[4] Needham and Pillai, 1937.

5. *3-Phosphoglyceric acid to 2-phosphoglyceric acid.* This phosphate-shift is catalysed by phosphoglyceromutase.



$$\begin{array}{l}\quad CH_2O\cdot H_2PO_3\\ \quad |\\ 2\;CHOH\\ \quad |\\ \quad COOH\end{array} \longrightarrow \begin{array}{l}\quad CH_2OH\\ \quad |\\ 2\;CHO\cdot H_2PO_3.\\ \quad |\\ \quad COOH\end{array}$$

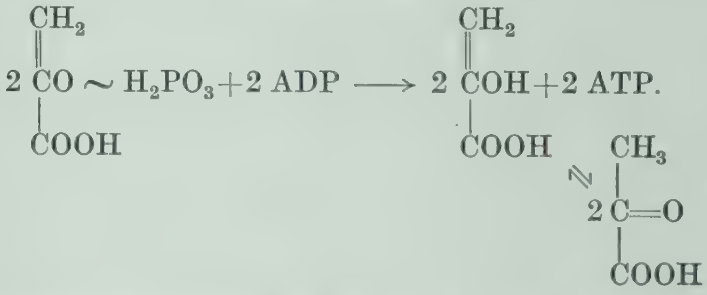
6. *Dehydration of 2-phosphoglyceric acid to* enol*phosphopyruvic acid.* The reaction generates a new energy-increasing linkage in place of the ester link at C-atom 2.



$$\begin{array}{l}\quad CH_2OH\\ \quad |\\ 2\;CHO\cdot H_2PO_3\\ \quad |\\ \quad COOH\end{array} \longrightarrow \begin{array}{l}\quad CH_2\\ \quad \|\\ 2\;CO\sim H_2PO_3+2\;H_2O.\\ \quad |\\ \quad COOH\end{array}$$

It is catalysed by enolase, which requires Mg^{++} ions and is probably a Mg-protein. It is not inhibited by dialysis; but is inhibited by M/40 sodium fluoride,[1] which, in the presence of phosphate, forms a complex magnesium fluorophosphate.[2] As a result, phosphoglyceric acids tend to accumulate when the reaction sequence is poisoned by fluoride.

7. *Dephosphorylation of* enol*phosphopyruvic acid* occurs in the presence of adenosine diphosphate with formation of ATP; that is to say the energy-rich phosphate of the *enol*phosphoric acid is transferred with further accumulation of available energy in ATP.



$$\begin{array}{l}\quad CH_2\\ \quad \|\\ 2\;CO\sim H_2PO_3+2\;ADP\\ \quad |\\ \quad COOH\end{array} \longrightarrow \begin{array}{l}\quad CH_2\\ \quad \|\\ 2\;COH+2\;ATP.\\ \quad |\\ \quad COOH\end{array} \rightleftharpoons \begin{array}{l}\quad CH_3\\ \quad |\\ 2\;C{=}O\\ \quad |\\ \quad COOH\end{array}$$

The reaction may be stopped by dialysing away the ADP. The *enol*-pyruvic acid first formed tends to equilibrium with *keto*pyruvic acid; but it is not known which of the two forms is the reactant in the next stage. Addition of calcium carbonate to a fermenting mixture causes calcium pyruvate to accumulate,[3] and addition of β-naphthylamine gives its condensation product with pyruvic acid, α-methyl-β-naphthocinchoninic acid.[4] Pyruvic acid also accumulates if carboxylase, the enzyme of the next reaction, is inactivated, for example, by alkaline dialysis.

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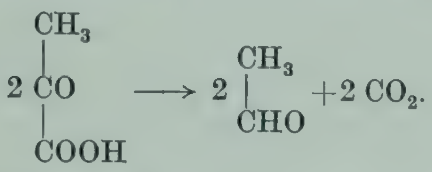
[1] See Meyerhof, 1935*a*.

[2] Warburg and Christian, 1942.

[3] Neuberg and Kobel, 1929.

[4] von Grab, 1921.

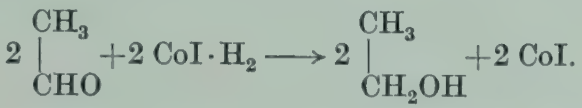
8. *Decarboxylation of pyruvic acid* is catalysed by carboxylase. The enzyme requires the presence of cocarboxylase (diphosphothiamine) and Mg^{++} ions. The coenzyme may be dephosphorylated by phosphatases so inactivating the enzyme. Aromatic sulphonic acids also inactivate.[1] The products of the reaction are acetaldehyde and CO_2.



$$2\,\begin{array}{c}CH_3\\|\\CO\\|\\COOH\end{array} \longrightarrow 2\,\begin{array}{c}CH_3\\|\\CHO\end{array} + 2\,CO_2.$$

All the previous reactions in the decomposition of hexosediphosphate are reversible; but the decarboxylation of pyruvic acid, with $\Delta F = -15{,}200$ cal is almost irreversible. It accounts for the total CO_2-release of fermentation. Acetaldehyde has been separated from fermenting mixtures by fixation with bisulphite and dimedon.[2]

9. *Reduction of acetaldehyde.* Acetaldehyde acts as H-acceptor from reduced Coenzyme I to give alcohol.



$$2\,\begin{array}{c}CH_3\\|\\CHO\end{array} + 2\,CoI\cdot H_2 \longrightarrow 2\,\begin{array}{c}CH_3\\|\\CH_2OH\end{array} + 2\,CoI.$$

The reaction is catalysed by alcohol dehydrogenase working in reverse, and is inactivated by iodoacetate[3] attacking —SH groups of the enzyme, or by bisulphite fixing the acetaldehyde. The oxidized CoI is again reduced by phosphoglyceraldehyde. In muscle, carboxylase being absent and lactic dehydrogenase present, pyruvic acid becomes the H-acceptor, and the reduction product is lactic acid. Since this does not involve the decarboxylation, all stages are reversible and triosephosphates, for example, have been obtained from lactic acid plus ATP by the use of muscle enzymes.[4] The net products of the normal fermentation of 1 mol. of hexose are thus 2 mol. CO_2, 2 mol. ethyl alcohol, and 2 energy-enriched phosphate molecules as ATP. In muscle glycolysis starting from glycogen, and so avoiding the utilization of one ATP, the net yield is three.

Induced formation of glycerol

The course of fermentation may be modified or diverted by the use of various metabolic blocks, some of which have been mentioned above. Two of the most important are associated with the name of Neuberg, and are known as Neuberg's second and third forms of fermentation respectively, the first form being the normal. Both lead to the accumulation of glycerol as principal product in place of alcohol.

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[1] Axmacher and Ludwig, 1936.

[2] Neuberg and Hirsch, 1919*b*.

[3] M. Dixon, 1937 (yeast); Berger and Avery, 1943*b* (oat coleoptile); alcohol dehydrogenase of animal tissues is not inhibited, Lutwak-Mann, 1938.

[4] Green, Needham, and Dewan, 1937.

Neuberg's second form of fermentation is brought about by addition of bisulphite to fermenting liquors.[1] This fixes the acetaldehyde formed in reaction (8) above, and prevents it from acting as H-acceptor. Under these conditions, 3-phosphoglyceraldehyde is in part oxidized by CoI and in part reduced by $CoI \cdot H_2$. The phosphoglycerol thus formed is hydrolysed by a phosphatase with release of glycerol and phosphate.

Neuberg's third form of fermentation is achieved by making the system alkaline and maintaining the alkalinity against the acid formation that then goes on.[2] In alkaline media, acetaldehyde instead of being reduced to alcohol is dismuted to a mixture of alcohol and acetic acid, and no longer gets its hydrogen from the phosphoglyceraldehyde. The result is again that the phosphoglyceraldehyde is in part reduced and hydrolysed to glycerol; but with a simultaneous formation of acetic acid and some alcohol. High yields of glycerol are obtainable from sugars by these methods. They were made an alternative source of glycerol for explo-

TABLE 28

Alcoholic fermentation by yeast

Reaction	*Product*	*Fixative*	*Enzyme*	*Coenzyme or carrier*	*Inhibitor*
1	triosephosphates	bisulphite HCN	zymohexase	. .	. .
2	1,3-phosphoglyceraldehyde	. .	spontaneous ?	(H_3PO_4)	. .
3	1,3-phosphoglyceric acid	. .	triosephosphate dehydrogenase	CoI	M/1000 iodoacetate
4	3-phosphoglyceric acid	. .	?	ADP	. .
5	2-phosphoglyceric acid	. .	phosphoglyceromutase	. .	. .
6	*enol*phosphopyruvic acid	. .	enolase	Mg	M/100 NaF
7	pyruvic acid	calcium carbonate, β-naphthylamine	?	ADP	. .
8	acetaldehyde $+CO_2$	bisulphite, dimedon	carboxylase	cocarboxylase	1/1,000 aromatic sulphonic acids
9	alcohol	. .	alcohol dehydrogenase	CoI	M/1,000 iodoacetate

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[1] Neuberg and Hirsch, 1919*b*.

[2] Ibid. 1919*a*.

sives in Germany during the 1914–18 war when fat-shortage had become acute.

The evidence for the similarity of glycolysis in the higher plants to the stages of alcoholic fermentation leans heavily on the action of fixatives and inhibitors. Their effects in fermentation are therefore summarized in Table 28.

GLYCOLYSIS IN HIGHER PLANTS

The occurrence and probable path of synthesis of fructofuranose-1, 6-diphosphate have been discussed on p. 111.

1. *The decomposition of hexosediphosphate* has been observed with mushes of peas and beans, with dried preparations and saps from fresh leaves of cigarette tobacco plants,[1] and with cleared saps from barley seedlings.[2] Zymohexase 'with a general resemblance to muscle zymohexase' was identified in potatoes. It was a stable enzyme, like zymohexase from other sources, and was not inhibited by iodoacetate, sodium fluoride, or cyanide.[3] It has since been identified in ferns, conifers, barley, and a wide range of other flowering plants, and is said to be most abundant in active tissues. In tomatoes and sugar beets it is located in the cytoplasm and absent from chloroplasts.[4] The products of its activity were first isolated after distillation with sulphuric acid as methylglyoxal (the hydrolysis product of triosephosphates) in the form of its *bis* 2,4-dinitrophenylhydrazone (m.p. = 298°).[1] The accumulation of unhydrolysed triosephosphate has been observed as an increase of alkali-labile phosphate in barley saps with added hexosediphosphate. The yield of triosephosphate was increased by addition of HCN or sodium bisulphite as aldehyde fixative; but only to a less extent by addition of iodoacetate to inhibit triosephosphate dehydrogenase. This is consistent with the known reversibility of the reaction.[2] Phosphotriose isomerase, catalysing the interconversion of the triosephosphates, has been identified in germinating peas.[5]

2–5. *Oxidation of triosephosphate to phosphoglyceric acid.* The stages of this oxidation have not yet been separated in higher plants; in particular it is not definitely known whether it is coupled with an additional phosphorylation as in yeast and muscle. An ester with the hydrolysis and solubility properties of phosphoglycerate is formed from hexosediphosphate by barley saps poisoned with sodium fluoride. This ester is not formed if the sap is poisoned with iodoacetate, which tends to arrest the reaction sequence at the triosephosphate stage.[2] Formation of a similar ester has been observed in spinach leaf *brei* incubated with glucose or glucose plus ATP.[1] The oxidation is carried out by triosephosphate dehydrogenase, which requires Coenzyme I as H-acceptor. The enzyme has been identified in germinating peas[2] and is probably present in *Lilium auratum* pollen[3] and in barley saps.[4] Coenzyme I is present in the spadix of *Sauromatum* to the extent of about 0·33 mg. in 1 gm. dried tissue[5] and has been found in traces in a variety of plants.[6] Addition of Coenzyme I to extracts from lily pollen[3] and barley saps[4] accelerates the oxidation of hexosediphosphate, presumably first glycolysed to triosephosphate in the usual way.

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[1] Neuberg and Kobel, 1934*a*, peas and beans; Baba, 1935, leaves.

[2] James, Heard, and James, 1944.

[3] Allen, 1938; see also Barron *et al.*, 1950.

[4] Stumpf, 1948; Tewfik and Stumpf, 1949.

[5] Stumpf, 1950.

6. *Consumption of phosphoglycerate* has been observed in barley leaves and *Scenedesmus*.[7] ^{14}C-labelled phosphoglycerate, generated by photosynthesis lasting 30 seconds, almost disappeared within an hour. There was simultaneous loss of $^{14}CO_2$.

7. *Formation of pyruvic acid.* More definite evidence of this stage is available, as pyruvic acid has been certainly identified and obtained from glycolytic precursors. Fresh *breis* or alcohol precipitates from germinating peas and beans have been incubated with sodium phosphoglycerate. After 41 hours at 37° C. they were deproteinated and precipitated with 2,4-dinitrophenylhydrazine. Yields of the pyruvic acid hydrazone were obtained corresponding with 20–35 per cent. of the phosphoglycerate added.[8] Similar experiments have been performed with barley saps to which 1-naphthol-2-sulphonic acid was added to restrict breakdown of the pyruvic acid by carboxylase. More elaborate methods were found necessary to isolate the pyruvic acid, which was obtained after 48 hours' incubation at 30° C. It was identified as its 2,4-dinitrophenylhydrazone (m.p. = 216° and other characteristics corresponding with a synthetic preparation). Yields of pyruvic acid were also obtained when hexosediphosphate was added to the saps instead of glycerophosphate; the transformation was inhibited by M/40 sodium fluoride. According to colorimetric estimation in an uninhibited sap, about one-third of the hexosediphosphate was recovered as pyruvic acid. The presence of sucrose or glucose did not cause pyruvate formation; but after the further addition of adenylic acid a phosphorylating system was set up, and pyruvic acid could again be isolated from the digests.[9] The glycolytic reactions actually identified in barley saps as leading to pyruvic acid formation are shown by the heavy arrows in Fig. 44.

Pyruvic acid does not appear to accumulate normally in plant tissues;

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[1] Bonner and Wildman, 1946.

[2] Stumpf, 1950.

[3] Okunuki, 1940.

[4] James, Heard, and James, 1944.

[5] van Herk, 1937.

[6] Whatley, 1951*a*.

[7] Calvin *et al.*, 1951.

[8] Neuberg and Kobel, 1934*b*.

[9] James, James, and Bunting, 1941.

but it has been isolated as the 2,4-dinitrophenylhydrazone (m.p. = 216°) from barley leaves poisoned with aromatic sulphonic acids.[1] Uninhibited leaves accumulated none, and its appearance only after the application of a carboxylase poison suggests a glycolytic origin. Pyruvic acid has also been obtained from onions;[2] but was probably

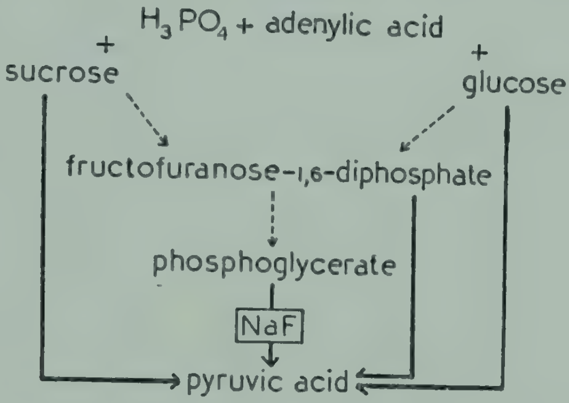
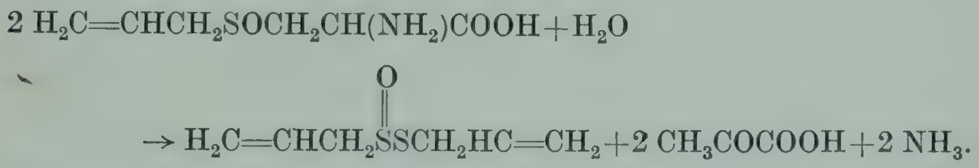


FIG. 44. Formation of pyruvic acid in barley saps.

not formed by their glycolysis. It is absent from the intact tissues and is formed rapidly during mincing; the formation is not inhibited by M/2 sodium fluoride.[3] Its most probable origin is from alliine, a constituent of onion oil, which is decomposed by alliinase to allicine, ammonia, and pyruvic acid.



$$2\,H_2C{=}CHCH_2SOCH_2CH(NH_2)COOH+H_2O$$
$$\rightarrow H_2C{=}CHCH_2\overset{O}{\overset{\|}{S}}SCH_2HC{=}CH_2+2\,CH_3COCOOH+2\,NH_3.$$

The extracted enzyme is extremely active.[4]

8. *Decarboxylation of pyruvic acid.* This reaction is also well authenticated in higher plants. Carboxylase, associated in suspensions with cell detritus, has now been extracted from a large number of them.[5] It has not been purified or obtained in solution. Its coenzyme, thiaminepyrophosphate, has also been recognized.[6] Inactivation by separation of the

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[1] G. M. James and W. O. James, 1940.

[2] Bennet, 1945.

[3] Morgan, 1946.

[4] Stoll and Seebéck, 1949.

[5] e.g. Zaleski, 1913, wheat seedlings, peas, beans, lupins, maize; Bodnar, 1916, potatoes and sugar beet; Bodnar and Hoffner, 1925, peas, wheat, and lupins; James and Norval, 1938, Bunting and James, 1941, barley seedlings; Horowitz and Heegaard, 1941, pea roots; Cohen, 1946, jack beans; Mee, 1949, soybean sprouts; Vennesland and Felsher, 1946, cucurbits, brassicas, Umbelliferae; Barron *et al.*, 1950, potatoes; Okunuki, 1939*a*, lily pollen.

[6] Tauber, 1937, onions, spinach, oranges, turnip shoots, &c.; Mee, 1949, soybean,

soluble cocarboxylase from the insoluble enzyme and reactivation by mixing have been carried out with barley,[1] soybean,[2] and cucurbit[3] preparations. The amount of cocarboxylase in barley seedlings was estimated as 30 μgm. per 100 gm. tissue.[4] In soybeans the amount was greater and increased rapidly on soaking, due apparently to phosphorylation of free thiamine; and slowly declined as germination proceeded. Increasing activity during soaking has also been observed with cucurbit seeds, and a corresponding slow decline during subsequent germination in barley seedlings. The activity of plant carboxylases is often very great, even comparing favourably with that of yeast.[3] Manganese has been used successfully to couple thiaminepyrophosphate and the apoenzyme.[2] It is not certainly known what metal normally forms the link in plant carboxylases; it is Mg in yeast.[5] The products of the isolated reaction with plant carboxylases have not been fully investigated. Acetaldehyde has been recognized on the basis of the sensitive and apparently specific Rimini variation of the nitroprusside reaction.[6] In dilute solutions, CO_2 may be liberated in amounts equivalent to the pyruvic acid supplied;[3] but there do not seem to be any corresponding determinations of acetaldehyde. The presence of oxygen does not inhibit acetaldehyde formation; but results in its partial condensation to acetoin (acetylmethylcarbinol) when a second enzyme, carboligase, is present.

$$2\,CH_3COCOOH \xrightarrow{\text{carboxylase}} 2\,CH_3CHO + 2\,CO_2$$

$$2\,CH_3CHO \xrightarrow[\text{carboligase}]{} CH_3CHOHCOCH_3.$$

This is what happens with yeast[7] and soybean[2] extracts. Formation is favoured by vigorous aeration or by the addition of methylene blue, suggesting that preventing the reduction of acetaldehyde by providing other H_2-acceptors (oxygen and methylene blue) favours the alternative condensation.[8] Acetoin is also known to be formed in small quantities in germinating barley.[9] It may be reduced to 2,3-butyleneglycol or oxidized to diacetyl; but there is no evidence that it undergoes either reaction; once formed in yeast it does not diminish.[10]

There have been numerous records of the slight accumulation of acetaldehyde in plant tissues both anaerobically and in air, usually on the basis of analytical results which lack specificity for critical identification.[1] A very interesting microchemical examination has been performed on sprouting seeds treated with a solution of *p*-nitrophenylhydrazine.[2] A crystalline hydrazone was obtained in small quantities from sprouting maize, *Secale cereale*, and *Oenothera biennis*. Air-dry and immature seeds gave no reaction. Although citrus fruits do not normally emit detectable acetaldehyde, either in nitrogen or in oxygen mixtures, considerable amounts are given off when they are first transferred from nitrogen to air. Acetaldehyde escaping under such conditions has been led into a solution of 2,4-dinitrophenylhydrazine and its hydrazone separated and identified by melting-point.[3]

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[1] Bunting and James, 1941.

[2] Mee, 1949.

[3] Vennesland and Felsher, 1946.

[4] Kinnersley in 1 above.

[5] Green, Herbert, and Subrahmanyan, 1941; Kubowitz and Lüttgens, 1941.

[6] Zaleski, 1913; James and Norval, 1938.

[7] Hirsch, 1922; Neuberg and Rosenthal, 1924; James and James, 1936.

[8] Kluyver, Donker, and Visser't Hooft, 1925.

[9] James and James, 1936.

[10] Whetham, 1927.

9. *Reduction of acetaldehyde.* The identification of ethyl alcohol in plant tissues kept under nitrogen has been described on p. 119. The enzyme, alcohol dehydrogenase, has been obtained, for example, from *Sauromatum* spadix,[4] lily pollen,[5] oat coleoptiles,[6] and peas.[7] The lily, oat, and pea enzymes have been shown, like the yeast enzyme, to require Coenzyme I. The oat enzyme is also known to be strongly inhibited by iodoacetate.[6] Apples and oranges, subjected to relatively high concentrations of acetaldehyde vapour, have been shown to form alcohol even in air and, at the same time, to decrease their sugar consumption.[8]

7*a*. *Reduction of pyruvic acid.* The identification of lactic acid in plant tissues is described on p. 123; but the circumstances of its formation do not point clearly to a reduction of pyruvic acid. The enzyme necessary for this reaction, lactic dehydrogenase, has been obtained from barley leaves[9] and lily pollen.[5] The activity was weak and could not be discovered in oat coleoptiles.[6]

Although there is not the same wealth of detail available for the higher plants that there is for yeast and muscle, this brief summary points to the essential similarity of glycolysis in all three. The following important identities appear to be reasonably well established. Glycolysis is catalysed in each by phosphorylation; the first rupture of the C-chain occurs when it is in the strained configuration of fructofuranose-1,6-diphosphate; triosephosphate is formed, and is oxidized by a dehydrogenase transferring the hydrogen to Coenzyme I; the first product after dephosphorylation is pyruvic acid. In plants in the absence of oxygen, pyruvic acid is mainly decarboxylated as in yeast;

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[1] e.g. Kostychev, Hubbenet, and Schelumov, 1913, poplar catkins; Kidd, West, and Trout, 1931, pears; Fidler, 1934, apples; Gustafson, 1934, tomatoes; Phillips, 1947, barley.

[2] Niethammer, 1929.

[3] Biale and Shepherd, 1939.

[4] van Herk, 1937.

[5] Okunuki, 1939*a*.

[6] Berger and Avery, 1943*a* and *b*.

[7] Adler and Sreenivasaya, 1937.

[8] Trout and Kidd, 1931; Fidler, 1935.

[9] James and Cragg, 1943.

but may, at least in some tissues, act as hydrogen acceptor as in muscle, and be reduced to lactic acid. The principal anaerobic H_2-acceptor of yeast and the higher plants is acetaldehyde giving rise to ethyl alcohol.

NATURAL ANAEROBIOSIS

In the main, lack of oxygen is something that happens to plants in laboratories, and its occurrence in the field is rather rare. Moreover, the tolerance by most plants of a strict anaerobiosis is limited. Dormant seeds, by virtue of their generally reduced metabolic needs, may survive long periods without oxygen; but active tissues usually show an early reduction of CO_2-emission followed by a general collapse. The accumulation of toxic products, such as alcohol, may be a contributory cause.

The rise of the water table round the roots of plants during floods may kill the root system up to the level reached by the water.[1] Stagnant water pools and swamps and the rooting medium below them provide the commonest anaerobic sites; but the plants inhabiting them may not endure anaerobiosis, but rather evade it. They frequently possess highly developed aerating systems of intercellular spaces, continuous from leaf to root, in which positive gas pressures may develop as a result of photosynthesis. The accumulating gas is largely oxygen.[2] In darkness the internal gas pressure falls as the oxygen is consumed and the more soluble CO_2 diffuses away.[3] Plants whose shoots reach the air may supply oxygen to the roots by similar morphological adaptations, the well-known pneumatophores of mangroves being a sensational special case. Even species without conspicuous aerenchyma may have narrow intercellular passages running the length of the root. These are easily observed in submerged barley roots, and may be the means by which those of oaks live in blue clays. However, it evidently will not do to jump to conclusions about their efficacy, as the submerged rhizomes of *Nuphar advenum* have been shown to accumulate alcohol while their floating leaves had none. Experimentally, the rhizomes were found to form alcohol in atmospheres with 3 per cent. oxygen or less.[4] It has also been claimed that the interior wood of trees may suffer an oxygen deficiency and may even form alcohol.[5] Analyses of the internal gas of ageing papaws have shown a sharp drop at the climacteric, associated with an increasing skin resistance to diffusion;[6] and there is a rather similar drift in bananas[7] and apples, in which an associated alcohol formation has also been demonstrated.[1] In these fruits an internal anaerobiosis seems to be induced, even in air, by a progressive proofing of the surface (cf. p. 150). As long ago as 1896, Gerber drew attention to this phenomenon, and suggested that it might provide the alcohol in the esters responsible for the aromas of ripening. Regarded strictly from the side of metabolic physiology it is a phenomenon of senescence and approaching death.

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[1] Balls, 1919, cotton plants in Egypt.

[2] According to Van Tieghem, 1866, about 90 per cent.; but according to Kniep, 1915, only 22·8–40·2 per cent. of the gas mixture escaping during photosynthesis is oxygen.

[3] For a general account see Arber, 1920.

[4] Laing, 1940.

[5] Devaux, 1899.

[6] Wardlaw and Leonard, 1936.

[7] Wardlaw and Leonard, 1940.

Many, if not all, seeds experience a brief anaerobiosis in the earliest stages of germination, especially if the seed-bed is very wet (p. 92). Typical products of anaerobiosis, such as lactic acid (p. 123), may temporarily accumulate. Normally the period of oxygen lack is very short, perhaps only a few hours, as the seeds swell and a normal oxygen uptake is restored. This is perhaps the most widespread type of natural anaerobiosis among plants, and is taken by the seeds in their stride; there is no arrest of germination and no obvious injury to the tissues. Longer deprivation may stop germination altogether.

Rice occupies an exceptional position, and its seedlings come nearest to the conception of a true facultative anaerobe among the higher plants. It has the reputation of germinating as well under water as in air, and, at high temperatures, even better.[2] Germination can occur in the complete absence of oxygen. In a comparison of rice with wheat grains,[3] an 88 per cent. germination of rice was obtained under nitrogen, whereas wheat would not germinate at all. An 80 per cent. germination of rice has been obtained under a 14 cm. depth of water.[4] It may be supposed that other water and swamp plants not yet examined have similar powers; but they are not universal. The reedmace, *Typha latifolia*, germinates well under reduced oxygen pressure; but in complete absence of oxygen does not germinate at all.[5] Rice under nitrogen produces CO_2 vigorously, and, in the earlier stages, alcohol accumulates in equivalent amounts. The rice plant's exceptional powers of anaerobic germination appear to be associated with an exceptionally vigorous anaerobic respiration.[6]

The early investigators, having in mind the existence of obligate anaerobes like the *Clostridia*, saw in anaerobic respiration a valuable adaptation allowing the colonization of exceptional sites and the temporary tolerance of exceptional conditions. Thus Pfeffer, 'all the reactions and properties which are indissolubly connected with the power of exhibiting vital activity are retained so long as intramolecular' (i.e. anaerobic) 'respiration continues'. Kostychev in 1927 rejected this position on two grounds. In the first place he contended that the release of energy in anaerobic respiration was too slight to provide for the plant's requirements, and was incapable of delaying the death of the plant, or of tiding its tissues over, even for a short time. Actually, this is unlikely to be true; plants suffer under nitrogen, not because their energy turnover becomes too small, but because it can no longer be harnessed. Secondly, Kostychev objected that the introduction of teleological considerations is an inadequate method of attacking biochemical questions. This was a local echo of Bacon's[1] more general rejection,

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[1] Hackney, 1943*a*; Fidler, 1933*a*.

[2] Akamine, see Lehmann and Aichele, 1931.

[3] Taylor, 1942.

[4] Valeton, 1907, cited from Lehmann and Aichele, 1931.

[5] Morinaga, 1926.

[6] Taylor, 1942; Phillips, 1947.

> For the handling of final causes, mixed with the rest in physical enquiries, hath intercepted the severe and diligent enquiry of all real and physical causes and given men the occasion to stay upon these satisfactory and specious causes, to the great arrest and prejudice of further discovery. . . . For to say that 'the hairs of the eyelids are for a quickset and fence about the sight' . . . or that 'the leaves of trees are for protecting the fruit,' or that 'the clouds are for watering the earth,' or that 'the solidness of the earth is for the station and mansion of living creatures,' and the like, is well enquired and collected in metaphysic, but in physic they are impertinent.

To decide whether anaerobic respiration is, or is not, the reason why plants do not die instantly upon being deprived of oxygen does nothing to explain within a scientific context either its origin or nature. In spite of this, a student of plants may well inquire whether anaerobic respiration has an adaptational value; at the present time with present data he can only conclude that any such value must be very slight.

Kostychev was probably wrong in supposing that the energy turnover in anaerobic respiration was not great enough to cover a plant's requirements. Assuming that the aerobic and anaerobic processes approximate in their results to complete oxidation and alcoholic fermentation, the free energy decreases under biological conditions are about 710 and 72 kcal per mol. respectively.[2] Further, since the rate of sugar breakdown may be faster under nitrogen than in air, the relative rates of energy release may lie closer together. On the basis of Blackman's data for apples, the ratio would be 710 to 317. The anaerobic turnover is much in excess of any requirement that can be shown to exist in plants. Nevertheless, the value of anaerobic respiration to the higher plants as an energy producer seems very doubtful. Yeast itself is unable to grow under nitrogen beyond a concentration of 28×10^4 cells per cu. mm. even though fermentation continues briskly. If the culture already contains this number of cells when brought under nitrogen, there is no further increase.[3] In most organisms, although cell division can require only a minute expenditure of energy,[1] it will not occur anaerobically. In the higher plants, translocation of solutes,[2] rapid uptake of salts[3] and dyes,[4] some absorption of water,[5] synthesis of sucrose[6] from hexoses, protoplasmic streaming,[7] and germination[8] all fail in the absence of oxygen or under the influence of oxidase poisons. It is even doubtful whether the functional condition of most plant tissues can be maintained anaerobically for more than a very short time. The protoplasmic structure of young barley leaves placed under nitrogen breaks down so completely that the cell contents ooze out within the first 48 hours, and before there has been any visible yellowing. This effect is exactly repeated if oxidation is inhibited by dilute cyanide,[8] and is greatly different from the normal progress of starvation in air. Without oxygen consumption, deterioration of the protoplasm appears to set in from the start; there is no true maintenance in its absence, only a measurable rate of breakdown.

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[1] *Advancement of Learning.*

[2] Wohl and James, 1942.

[3] Brown, 1892.

These various results indicate that anaerobiosis is not a way of life for higher plants, and that its energy transformations fail to maintain plant activities, not through being too meagre, but because the appropriate mechanism for harnessing the energy does not exist. Even though oxygen is not involved as a direct reactant, it is needed to set in train the reactions which link sugar degradation with the processes increasing free energy.

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[1] Gray, 1931.

[2] Mason and Phillis, 1936.

[3] Steward, 1935; Steward, Berry, and Broyer, 1936.

[4] Wohl and James, 1942.

[5] Reinders, 1938.

[6] McReady and Hassid, 1941; Nelson and Auchincloss, 1933.

[7] Ewart, 1903.

[8] James and Hora, 1940.

VII

OXYGEN EFFECTS

PLANT tissues are supplied with oxygen by diffusion. This is a slow process, even in a gas phase; but is very much slower if the diffusion path lies through a liquid. The rate of gaseous diffusion of oxygen is of the order 10^4 times faster than its rate of diffusion through water, other things being equal. With the introduction of a liquid, solubility also becomes a factor, and the supply of a gas of low solubility like oxygen may be greatly restricted, with consequences such as those described for seedlings on p. 92. The plant body itself is about 85 per cent. water, and it might seem that access of oxygen to the interior of a bulky tissue would, on this account, be severely limited. Many tissues are freely penetrated by intercellular spaces which in woody stems, for example, are in continuous connexion from the surface to the heartwood, even through the cambium. Furthermore, cells with thickened walls often show pits facing the intercellular spaces in which the respiring cytoplasm is brought closer to the gas phase,[1] and the general anatomical study of plants affords numerous examples of structures well adapted for aeration.[2] Bearing in mind the slow rates of plant respiration and the relatively high concentration of oxygen in the outside air, the existence of intercellular-space systems must do much to ensure normal plant tissues (as distinct from the exceptions discussed at the end of the last chapter) from oxygen lack. Nevertheless, it is well known that their porosity varies widely, from the spaces forming the bulk of the aerenchyma of many swamp plants to the minute openings in potatoes estimated at about 1 per cent. of their total volume.[3] In addition, to use the words of Haberlandt, it would be far from correct 'to assume that there is always a close correspondence between the average intensity of respiration and the degree of development of the ventilating spaces'. It is therefore desirable to have some estimate of the volume of tissue that may exist without benefit of intercellular aeration.

OXYGEN INVASION OF BULKY TISSUES

The rate of entry of gas into a liquid body depends on its shape, i.e. on the amount of surface developed. The simplest form is an unlimited flat plate with entry at both surfaces. Assuming a uniform concentration of gas to be maintained at the surfaces, Warburg (1923) developed the equation

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[1] Strasburger, 1891; James and Baker, 1933.

[2] See, e.g. Eames and Macdaniells, 1947; Arber, 1920, for water plants; and especially Haberlandt, 1914, for a systematic description of the 'ventilating system'.

[3] Burton and Spragg, 1950; Burton, 1950.

$$U = C - \frac{ax(H-x)}{2D}$$

where U = oxygen concentration, expressed in atmospheres, at distance x cm. from the surface; C = external oxygen pressure in atmospheres; a = oxygen consumption in ml. (N.T.P.) per min. per ml. tissue; H = thickness of tissue in cm.; and D = invasion coefficient, or ml. (N.T.P.) per sq. cm. per min. with gradient 1 atm. per cm. A uniform rate of respiration throughout the tissue is assumed. U and D in the above expression are both given as partial pressures in a gas phase with which the solution is equilibrated. This method was introduced by Krogh (1919) and has advantages, notably that the invasion coefficient thus expressed is virtually constant over the biological range of temperatures, altering only 1 per cent. per degree above or below 20° C. This results because it covers both solubility and diffusion which are affected by temperature in opposite senses. Invasion coefficients are sometimes referred to as diffusion coefficients, which seems liable to cause confusion. The units used by Krogh are retained here to mark the difference from true diffusion coefficients which are measured in C.G.S. units; to convert to C.G.S. units divide by 60.

Warburg's equation may be applied to fronds of *Ulva* taking an average thickness of 34×10^{-6} cm. and a respiration rate $8{\cdot}7 \times 10^{-3}$ ml. O_2/min./ml. tissue.[1] Assuming $D = 3{\cdot}4 \times 10^{-5}$, the value for water, i.e. that surface membranes do not seriously interfere, the oxygen concentration in the central line of the frond would be 0·19 atm. and almost identical with the value of 0·2 atm. at the surface.

To calculate the maximal thickness of a plate of tissue that can be associated with a given respiration rate, since x then equals $H/2$, the equation may be transposed to

$$H = \sqrt{\left(\frac{8D(C-U)}{a}\right)}.$$

Taking 2 per cent. O_2 as an average requirement for the avoidance of toxic anaerobiosis and 10^{-2} ml. O_2/min./ml. tissue as a moderate respiration rate (cf. *Ulva* and its position in Table 1), $H = 0{\cdot}45$ mm. This would be the limiting thickness of laminar organisms, without internal circulation or aeration, and with moderate respiration requirements. It is about 13 times the thickness of *Ulva*, which is unlikely, therefore, to suffer from restriction of oxygen supply for its respiration.

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[1] Goddard, 1946.

Invasion of oxygen into tissue slices

Disks of storage tissue have been very favourite material for respiration experiments, carrots affording perhaps the most used. Their air space has been estimated at < 3 per cent. of the tissue volume;[1] but it appears to be very differently arranged from that of potatoes. According to Briggs and Robertson it is limited more or less completely to the corners of the cells. On the basis of small cubes (air spaces) arranged at the corners of otherwise cubical cells, they calculate that 4·3 per cent. gas phase would increase the coefficient of diffusion by only 6·6 per cent. Maximal respiration rates for carrot disks are given as equivalent to $9{\cdot}33 \times 10^{-4}$ ml./min./ml. tissue.[2] For 5 per cent. oxygen concentration at the centre plane, Warburg's equation (p. 143) gives a limiting thickness of 0·216 cm., or for zero oxygen at the centre 0·24 cm. Turner, calculating from unspecified data, gives 0·3 cm. as the permissible limit and 0·1 cm. as a convenient working thickness. Briggs and Robertson, using the same equation with their own carrot data, give a thickness of 0·2 cm. for an oxygen pressure of 2·5 per cent. at the centre. Liver slices have much faster respiration rates (Table 1) and lower invasion coefficients, and Dixon (1934) gives a limiting thickness as low as 0·021 cm. Such thin slices are too fragile for shaking in a respirometer and, since thicker ones must be used, the external oxygen concentration must be raised; it is usual to replace the air in the experimental vessels by oxygen. Owing to their more sluggish respiration, it is not necessary to do this with plant tissue slices, and the oxygen concentration may even be reduced below the air value. To maintain a central concentration of 2 per cent. oxygen in 0·1 cm., carrot slices respiring at the rate already given would require an external concentration of only 5·4 per cent. If the resistance of the tissue to oxygen entry is considerably above that of water, this figure would have to be raised; but there is no evidence of this for plant tissues. According to the measurements of Briggs and Robertson the invasion coefficient of CO_2 into carrot disks at 21° C. corresponds to $1{\cdot}02 \times 10^{-3}$, and the corresponding figure for water at 20° C. is $1{\cdot}06 \times 10^{-3}$. The same agreement may be expected to hold for oxygen. Krogh gives invasion coefficients for oxygen of $3{\cdot}4 \times 10^{-5}$ into water, $2{\cdot}8 \times 10^{-5}$ into gelatine, and $1{\cdot}4 \times 10^{-5}$ into muscle; the water value has been taken in the foregoing calculations.

From the above figures for carrot slices, it might be supposed that oxygen diffusion would play a conspicuous part in limiting the aerobic respiration of tissues of quite moderate thickness. Actually, these fragments of tissue with their induced respirations are not characteristic in this respect of the unbroken organ. Choudhury (1939) measured the CO_2-output of whole carrots of unspecified diameter, but of normal market size, and obtained a result equivalent to $1{\cdot}7 \times 10^{-4}$ in Krogh's units, i.e. less than a fifth of the rate recorded for 0·1 cm. disks. Assuming a cylindrical form, we may apply Fenn's equation (p. 144) to find the radius at which this rate would reduce the oxygen concentration at the centre of the whole carrot to zero. It works out at only 0·40 cm. or 0·32 cm. for 5 per cent. oxygen at the centre, at which concentration alcohol formation is likely to begin.[1] Choudhury's results show, however, that carrot respiration rates fall off rapidly in oxygen concentrations below atmospheric; consequently the oxygenated radii are increased to unknown limits. It also seems possible that the effect of the intercellular spaces has been underestimated. Briggs and Robertson noted that those of carrot disks suspended in unaerated water became water-injected, and it is difficult to see why this should happen if the spaces were limited strictly to the corners of cells and hence discontinuous.

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[1] Briggs and Robertson, 1948.

[2] J. S. Turner, 1938*a*. Similar values are given by Stiles and Dent, 1947; Briggs and Robertson, 1948.

THE RELATION BETWEEN OXYGEN UPTAKE AND OXYGEN CONCENTRATION

Owing to the preoccupation of the early plant physiologists with carbon dioxide measurements, relatively few data have been obtained for oxygen uptake under varying conditions. The most exact data at present available are from Watson's (1932) experiments with apples; these experiments provide a careful analysis of the relation of consumption to concentration; and a number of other investigations, usually undertaken with other primary objects in view, give general confirmation.[2] In Watson's experiments Bramley's Seedling apples were maintained in an air flow at 22° C., and the oxygen removal measured by gas analysis of successive samples. After a steady rate of respiration had been established, air was replaced by successive gas mixtures, and a characteristic steady rate was obtained for each mixture, usually after 40 hours. The experiment was finished by passing air for a second time. It was not possible to examine more than 3 or 4 gas mixtures in addition to air with a single apple; and the final rate in air was always found to differ somewhat from the initial. To obtain a unified statement of the results, the air-line was drawn between the steady initial and final values, and the rate of oxygen consumption in each gas mixture expressed as the ratio between its steady value and the simultaneous value given by the interpolated air-line. The rate of oxygen consumption in air, thus recorded as unity, varied round 2 mg./hr./100 gm. apple. The results are shown in Fig. 45. On test by plotting $1/R$ against $1/[O_2]$, the curve is found a good fit to a rectangular hyperbola. It is further notable that saturation had not been reached at the air value, where the curve was still obviously rising. Curves for rice,[1] barley seedlings,[2] and onion roots[3] show the same general features. The investigation of young growing seedlings is complicated by the rapid, spontaneous changes of their respiration rates and idiosyncrasies of the material. Forward has examined the respiration of barley seedlings in considerable detail and provides a summarizing curve of the relationship (Fig. 46). Respiration rates were again calculated in terms of the air value; but the ratio was obtained in a different manner. After a transition from air to an oxygen-nitrogen mixture, the final value in air was compared with the initial rate in the mixture instead of with its adjusted state. In spite of this difference the curve shows remarkable similarity to Watson's, and the author comments that it approaches a

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[1] Carrot respiration is strongly anaerobic at 2·5 per cent. oxygen. J. S. Turner, 1938*b*.

[2] Platenius, 1940, asparagus, apples, and peas; Vlamis and Davis, 1943, rice and barley seedlings; Rosene, 1950, onion roots.

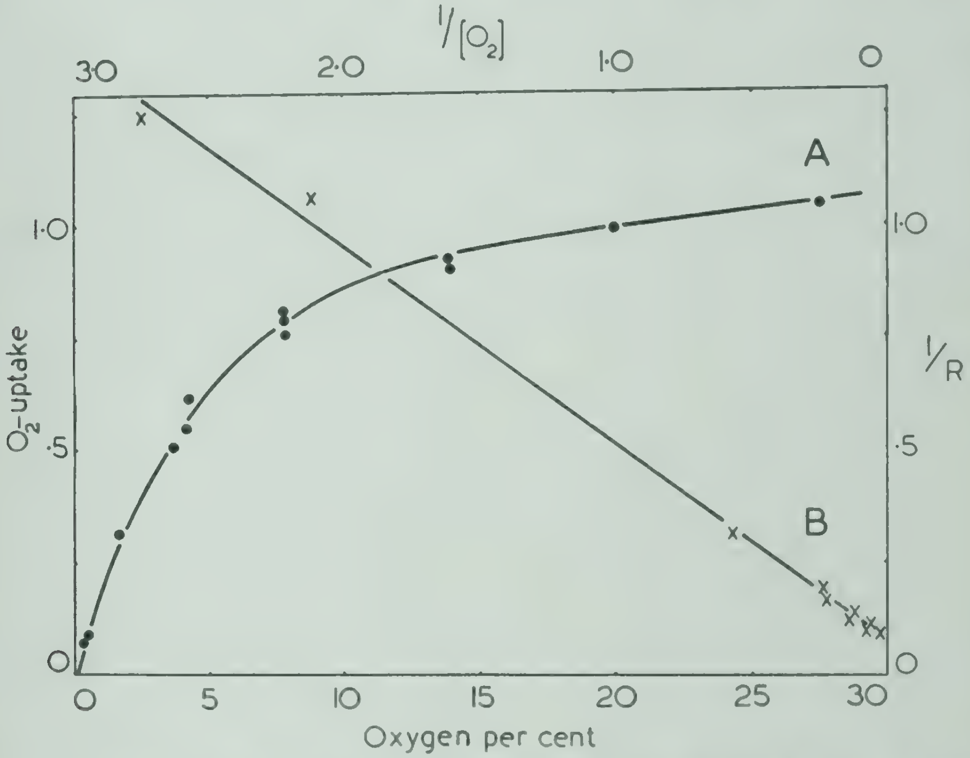

FIG. 45. Curve A. Oxygen consumption (R) of Bramley's Seedling apples at various oxygen concentrations. Rate in air = 1·0. Data of Watson. Curve B. $1/R$ plotted against $1/[O_2]$ to show hyperbolic form of curve A.

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[1] Vlamis and Davis, 1943.

[2] Vlamis and Davis, 1943; Forward, 1951.

[3] Rosene, 1950.

rectangular hyperbola and, further, that the divergence found, on test, in very low oxygen concentrations, is likely to be due to a significant fall in the external oxygen supply. It seems probable that an oxygen hyperbola occurs with a wide variety of organisms, including animals and bacteria.[1] Unfortunately it is a very generalized type of relationship and could arise from a number of causes. The most obvious is the possibility of its being due to the reaction of oxygen with a respiratory enzyme according to the Michaelis-Menten rule. If this were so, the form of the curve and the concentration for half-maximal velocity might be set by the affinity of the enzyme for its substrate, oxygen. But oxidases have a second substrate, the reductant, and the apparent oxygen affinity, $1/K_m$., falls slowly with rising concentration of reductant. The oxygen affinities of different enzymes may, however, differ by much greater amounts (Table 29, p. 165). The relation may be further complicated by the conditions of access of oxygen to the enzyme. A high resistance would not lower the maximum rate attainable, but would increase the oxygen concentration required for its achievement. In other words, an enzyme of high oxygen affinity, embedded in a tissue offering resistance to the entry of oxygen, might well need an oxygen concentration for half-maximal velocity similar to that needed by an

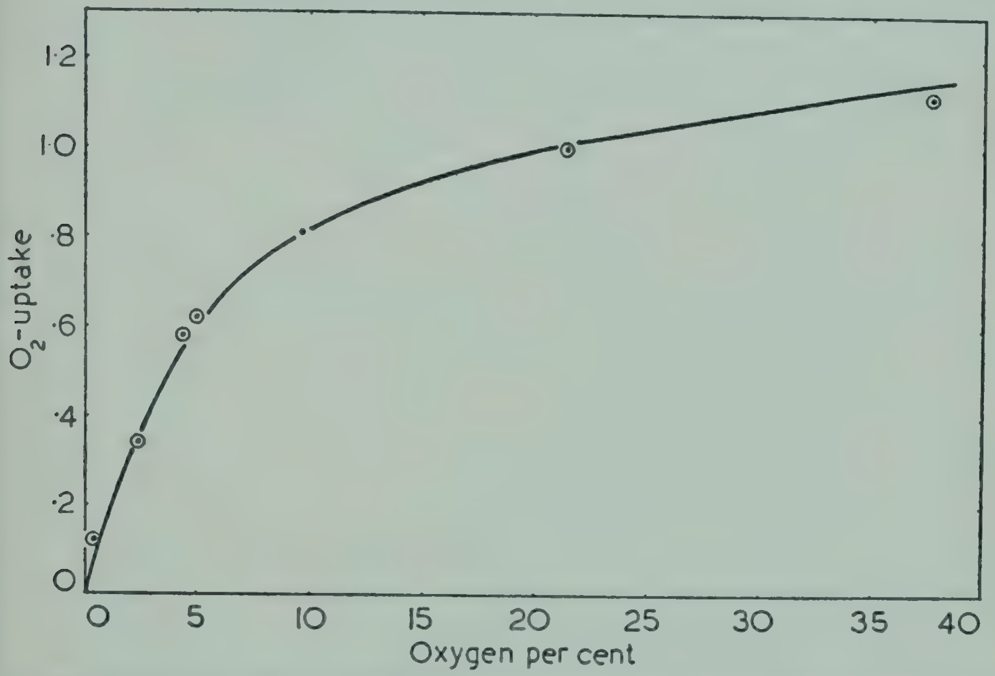

FIG. 46. Relation of oxygen uptake to concentration of oxygen entering the respiratory chamber with young barley seedlings. Uptake in air = 1·0. Circles are replicated observations. After Forward, 1951.

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[1] See, for example, Tang, 1934; Kempner, 1939.

enzyme of low oxygen affinity. These relations, with a fixed concentration of enzyme, are schematized in Fig. 47, curves A and B.

There is no reason to suppose that the results of Watson's experiment illustrated in Fig. 45 were diffusion-restricted to any great extent. They were performed with apples about to ripen (early senescence) with a moderate respiration rate. The flesh is an extremely porous tissue, and in Bramley's Seedlings about 28 per cent. of its volume is intercellular air space;[1] what resistance there is lies in the skin. Skin resistance appears to be at its minimum in preclimacteric apples, and to increase rapidly during late senescence. There is either a positive correlation or none between respiration rate and internal oxygen concentration at the earlier stages, and it is only later that a strong negative correlation develops.[2]

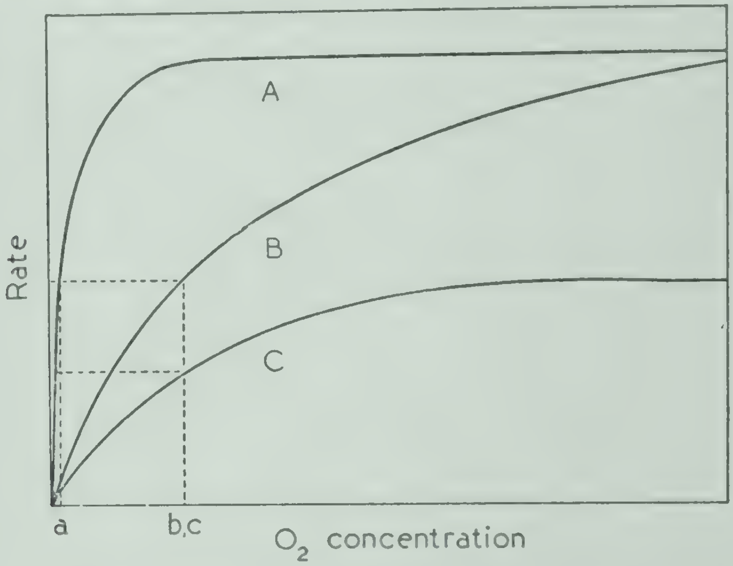

FIG. 47. Diagram of the relation between rate of oxygen uptake and oxygen concentration. Curve A, with enzyme of high oxygen affinity, and resistance to access of oxygen low. Curve B, high affinity and high resistance, or low affinity with low resistance. Curve C, as B but with less enzyme. *a*, *b*, *c*, concentrations for half maximal velocity.

In a further series of experiments Watson showed the effect of these changes on O_2-consumption. Small apples picked from the tree in July gave a normal hyperbolic curve (Fig. 48A); but others taken from store after the lapse of 8 months or more showed progressive depression of the curves at moderate oxygen concentrations, precisely as would be expected to result from the increasing skin resistance. The complete record is more complex than Fig. 48 indicates, since intermediate experiments between July and March showed lower values in air. This is typical of the normal respiratory drift of maturing apples and is not dependent on skin resistance, since at this stage low resistance is associated with low rates. Comparison of two sets of results[1] for rice (Fig. 48B) suggests that the earlier was strongly diffusion-restricted. Both methods of experimentation appeared adequate to secure a proper gas supply to the tissues; but the earlier results were obtained during the first few hours of germination, while the latter used seedlings that had already put out their shoots to a length of 2 cm. Corresponding results were obtained with wheat in the first investigation and with barley in the second. It is therefore interesting to notice that the diffusion-inhibited seedlings were at the stage for which a temporary high resistance to oxygen entry has already been inferred on other grounds (p. 92).

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[1] W. H. Smith, 1937.

[2] Hackney, 1943*a* and *b*; 1944*a* and *b*.

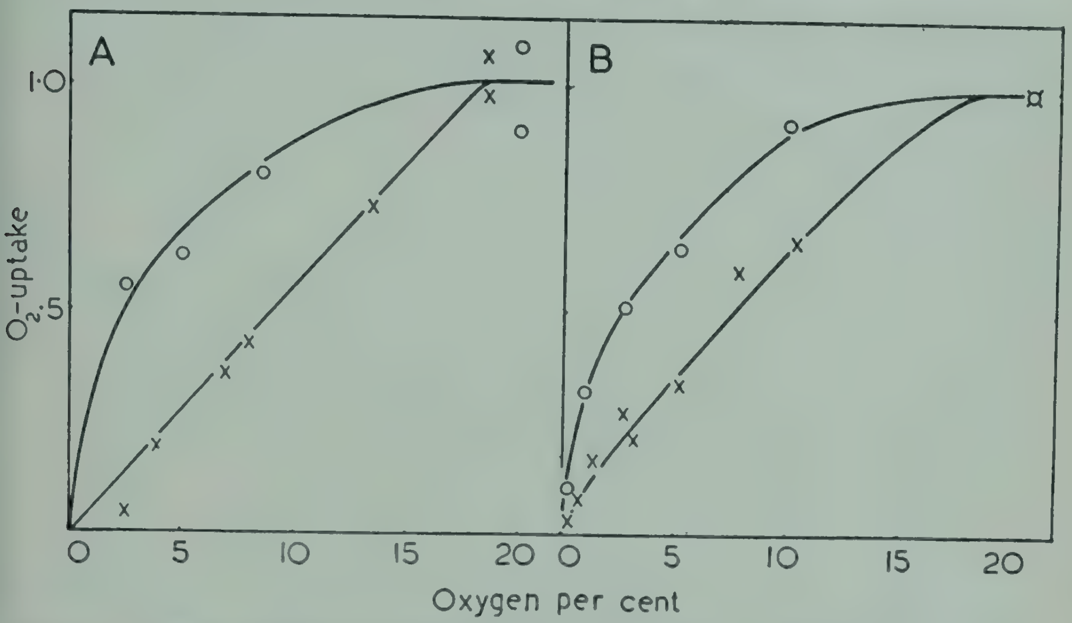

FIG. 48. Oxygen uptake at varying oxygen concentrations. Uptake in air = 1·0. A. Apples. ○, young apples just picked from the tree in July; ×, senescent apples of the same harvest twelve months later. Data of Watson. B. Germinating rice seedlings. ○, seedlings with 2-cm. shoots. Data of Vlamis and Davis. ×, seedlings just beginning germination. Data of Taylor.

It appears most unlikely that the oxygen-affinity of their oxidases sets the rate limit in apples (p. 166); but it plays an important part in the spadix of *Arum*. There is a strong contrast between the oxygen curves of its spadix and stalk tissues[2] as shown in Fig. 49. The O_2-uptake of spadix slices with air is about 20 times faster than the rate for stalk slices, so, to display the two graphs on a single diagram, they have both been calculated in terms of their own air values and therefore intersect at that point. It will be seen that the stalk tissue reaches oxygen-saturation at about 5 per cent. oxygen, whereas the spadix slices were still unsaturated at 100 per cent. (1 atm.). Owing to the very rapid rates of O_2-uptake by the spadix slices, it might be supposed that they

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[1] D. L. Taylor, 1942; Vlamis and Davis, 1943.

[2] James and Beevers, 1950.

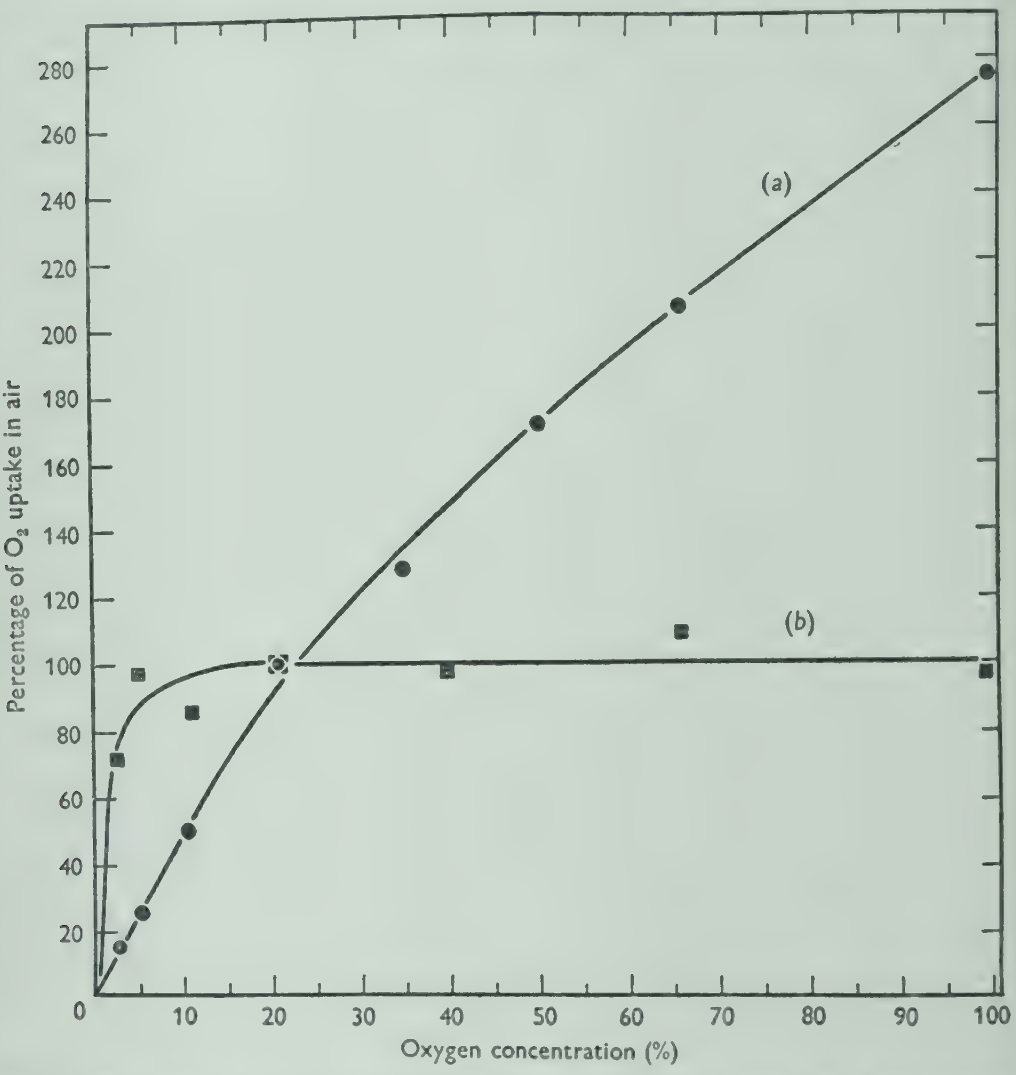

FIG. 49. The effect of oxygen concentration on the uptake of oxygen by *Arum maculatum*. (*a*) spadix slices, (*b*) stalk slices. The curves are plotted to coincide at the air value. James and Beevers, 1950.

were diffusion-restricted in the middle ranges. Calculation on the basis of the Warburg equation (p. 143) suggests that, if the intercellular spaces became waterlogged during the experiment, this might have occurred to some extent; but there was no reason to suspect injection as the material was kept fully aerated. Moreover, direct evidence was forthcoming that the respiratory oxidases were different in the two tissues. The stalk possessed a metallo-enzyme of polyphenol oxidase type, while the spadix contained no metallo-enzyme at all, and its respiration depended on an autoxidizable flavoprotein. At least some metallo-enzymes are known to have high oxygen-affinities[1] and some flavoproteins low ones[2] (p. 165).

In the examples cited partial explanations of the forms of the oxygen curves can be proposed; but it should not be assumed that such simple considerations will always be applicable or even that they elucidate fully the cases chosen.

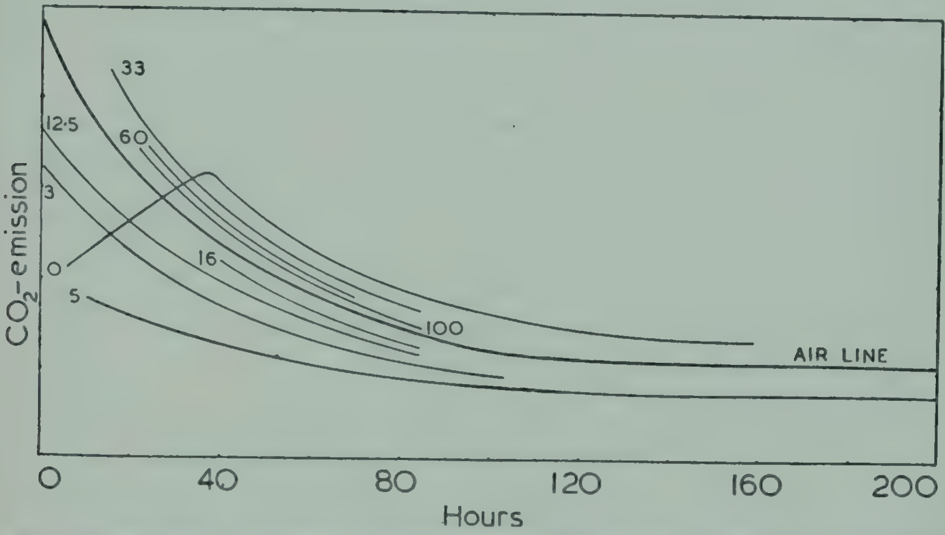

FIG. 50. CO_2-emission by cherry-laurel leaves kept in various concentrations of oxygen. From a diagram by F. F. Blackman using the data of Parija.

OXYGEN CONCENTRATION AND CO_2-PRODUCTION

The carbon dioxide curves show a feature that is not present in the oxygen curves just discussed. Though they follow a similar course at high oxygen concentrations, they do not proceed smoothly through low concentrations to the origin, but exhibit a minimum, usually somewhere within the range 2–5 per cent. O_2. At lower concentrations, the CO_2-emission may remain more or less constant or rise gently or steeply, so that in the absence of oxygen the rate of CO_2-emission may be greater or less than in air. Rates shown by a series of detached cherry-laurel leaves kept in various oxygen concentrations are shown in Fig. 50. The curves run parallel over long periods and the relative rates can be expressed by simple ratios; only in the complete absence of oxygen is the relation complicated by an initial period of adjustment before the curve settles, at least for a time, into a parallel course. The convention, already adopted with the O_2-consumption curves, of taking the air value as unity, is again used and the results are shown in Fig. 51. There is a well-marked minimum at 5 per cent. oxygen and a steep rise, reaching well above the air-value, in pure nitrogen.

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[1] Warburg and Kubowitz, 1929, found oxygen saturation of the cytochrome system at 10^{-3}–10^{-5} atm.

[2] See, for example, Green, 1940; Laser, 1952.

This is an alternative method of expressing results already stated in the form of R.Q.s (p. 86); since, when the CO_2-formation and O_2-uptake curves begin to diverge, the R.Q. will rise. Stich (p. 86) noted that a rise of the R.Q. began at about 3 per cent. oxygen with wheat, maize, and pea seedlings and Gustafson recorded a rise at 2 per cent. oxygen for red tomatoes. It is logical, therefore, to apply the same explanation, and to suppose that the CO_2-emission in low oxygen concentrations is of composite origin, partly aerobic and partly anaerobic. Even before suitable oxygen data were available, F. F. Blackman suggested that the CO_2-curve for cherry-laurel (Fig. 51) could be regarded as the resultant of aerobic and anaerobic components. As a simple hypothesis, it was assumed that the extinction of anaerobic respiration was proportional to oxygen concentration, i.e. that the anaerobic CO_2-emission was represented by a straight line descending from the full anaerobic value, to cut the abscissa axis at a finite oxygen concentration which was named the extinction point. In the absence of O_2-absorption rates, the choice of slope for the anaerobic curve becomes more or less arbitrary; and it is

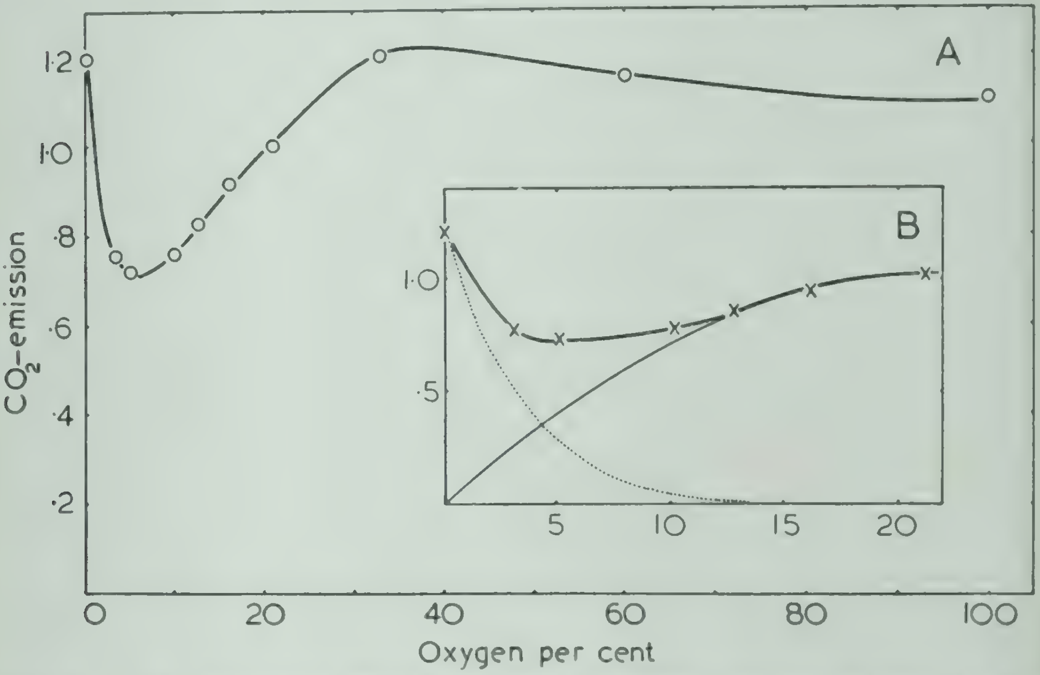

FIG. 51. A. CO_2-emission of cherry-laurel leaves at oxygen concentrations from 0 to 100 per cent. Data of Parija. B. (inset) Initial part of curve with expanded abscissa axis. Aerobic CO_2 indicated below 12·5 per cent. oxygen as smooth hyperbolic continuation of curve above 12·5 per cent.; anaerobic CO_2 (dotted line) by difference.

readily found on trial that to represent the anaerobic contribution by a straight line results in an irregular and most improbable curve for the residual aerobic fraction.

The relation was more fully investigated in Blackman's laboratory by Watson who, in the apple experiments already mentioned, determined CO_2-emission as well as O_2-uptake. The two curves are plotted together in Fig. 52. Above 15 per cent. oxygen they run together, but below this they diverge progressively. Assuming that the R.Q. of aerobic respiration remains the same at all oxygen concentrations, the aerobic CO_2-output is easily calculated and the anaerobic obtained by difference. This is shown as a dotted line in Fig. 52. On the basis of Watson's results, it is now possible to make a more satisfactory analysis of the cherry-laurel curve as well. The aerobic curve may be drawn as a smooth hyperbola from the 12–15 per cent. oxygen values back to the origin; and the anaerobic values again obtained by difference (Fig. 51B).

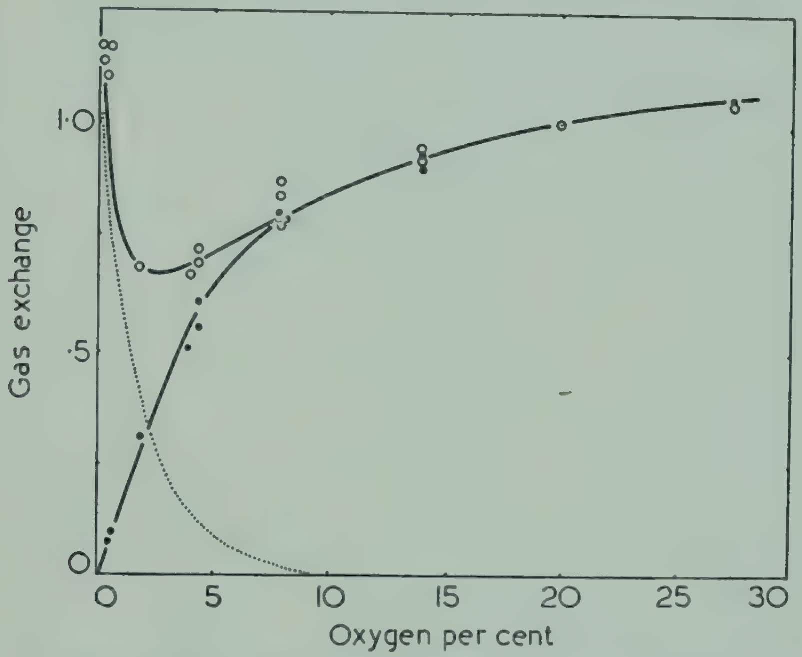

FIG. 52. Gas exchange of Bramley's Seedling apples at various oxygen concentrations. Rate in air = 1·0. ●, O_2-uptake; ○, CO_2-emission observed. Dotted line shows the anaerobic CO_2. After Watson.

Similar die-away curves are then given by both materials, anaerobic respiration being imperceptible above about 10 per cent. oxygen. The inhibitory effect of oxygen is represented by the complementary curves with an asymptote numerically equal to the fully anaerobic value (Fig. 53). They are fairly close approximations to rectangular hyperbolas, the relation to be expected if oxygen were inhibiting by combining with an enzyme, or a continually renewed intermediate product, controlling the rate of anaerobic CO_2 production. Oxygen itself is unlikely to react with either directly, but the same relation may hold if it operates through an oxidation system. If the oxidase concerned has the usual high affinity for oxygen, the course of the inhibition curve must be affected by some other factor, perhaps a moderately high diffusion resistance.

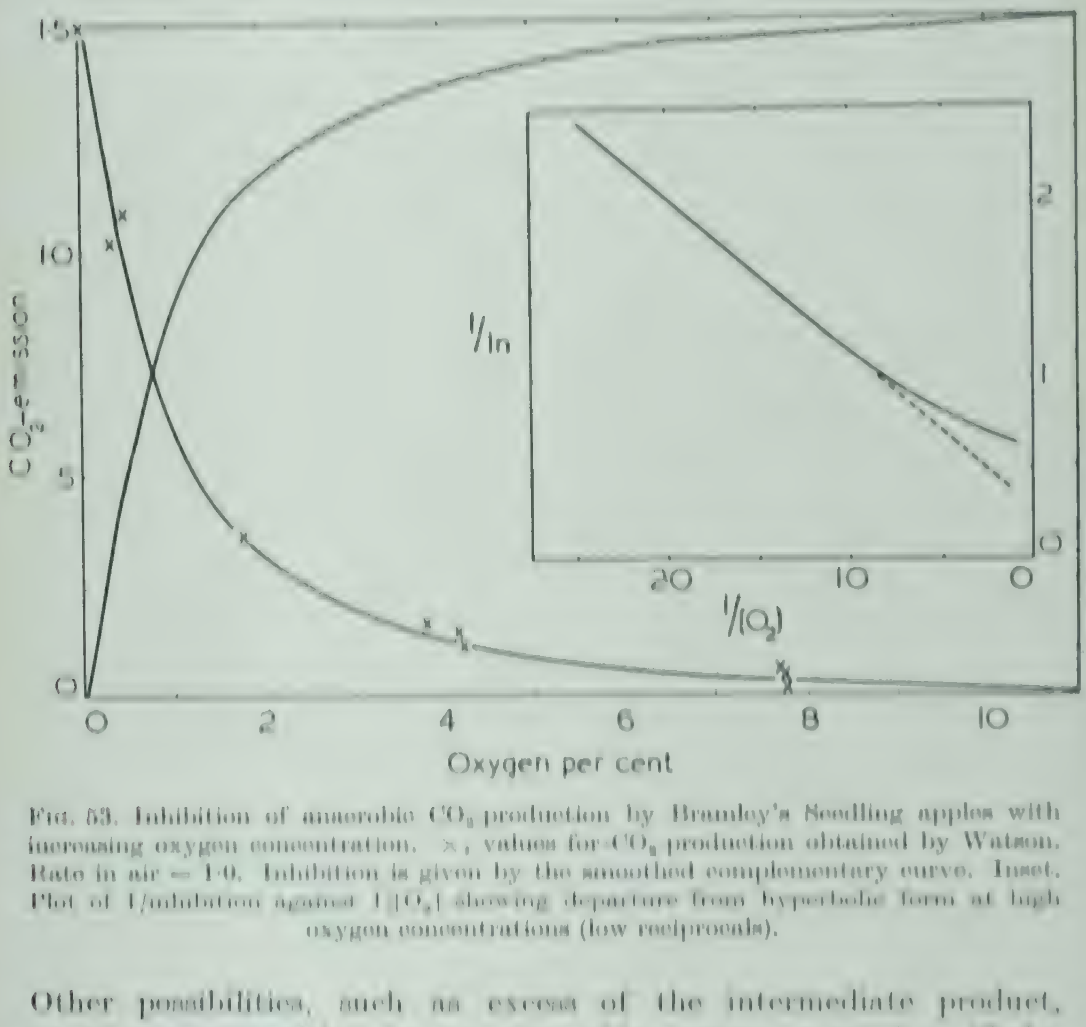

FIG. 53. Inhibition of anaerobic CO_2 production by Bramley's Seedling apples with increasing oxygen concentration. ×, values for CO_2 production obtained by Watson. Rate in air = 1·0. Inhibition is given by the smoothed complementary curve. Inset. Plot of 1/inhibition against $1/[O_2]$ showing departure from hyperbolic form at high oxygen concentrations (low reciprocals).

Other possibilities, such as excess of the intermediate product, or strong competition by an anaerobic system, seem less likely. Although the curves are not far from being true hyperbolas, the relation is found, on test, to fail in the higher oxygen range, where the inhibition falls below prediction. This would be consistent with the suggestion that surplus oxygen, not consumed in aerobic respiration and accumulating in the tissues, increases the rate of supply of the controlling substance. This is also suggested by Watson's observation that, after a transfer from nitrogen to air, O_2 consumption and CO_2 output continued to rise long after steady diffusion conditions were set up. Such an hypothesis was first put forward by F. F. Blackman on somewhat different grounds (p. 164).

An alternative explanation of the die-away curves might be derived from statistical considerations. Apples consist of many cells, and it might be that extinction points of individual cells varied over a considerable range. Almost all the CO_2-output of an apple comes from the skin and flesh which respire at about the same rate per cell.[1] Within each tissue the cell type is fairly homogeneous and variation of cellular extinction points would be expected to have normal distribution. On this view, suppression of anaerobic CO_2-output would increase in sigmoid progression with oxygen concentration; but this is contrary to the data, the curve of inhibition in Fig. 53 having no hollow at low oxygen concentrations. We therefore reject a statistical explanation in favour of the metabolic one already given.

Extinction point and extinction coefficient

The former name was given to the oxygen concentration at which anaerobic respiration is extinguished, since it was originally supposed that it was a finite and determinable point. The results of Watson make this appear very improbable. The suggestion sometimes made that the extinction of anaerobic respiration coincides with the inflexion point of the total CO_2-emission curve is also clearly inadmissible, even when the bend can be located within reasonable limits. Any attempt to draw the consequent curve for the aerobic component brings conviction on this point. If alcohol production is taken as an alternative measure of anaerobic respiration, the technical difficulties are greatly increased; but the experiments with apples of Thomas and Fidler (1933) showed that, where enough points were available, the die-away curves were also gradual over a similar range to Watson's (Fig. 55, p. 160).

Both sets of experiments agreed that the anaerobic respiration of apples advances into higher oxygen concentrations as the storage season proceeds. It is therefore desirable to have some method of expressing the slope of the curve. As a simple extinction coefficient, convenient for comparisons with enzyme data, we may propose the 'half-maximal' concentration, i.e. the oxygen concentration at which anaerobic CO_2-production is reduced to 50 per cent. of its full rate under nitrogen. At this point the curve is always steeply sloped, and the concentration can be determined closely. Thus, the coefficient for apples in early senescence, read from Fig. 52, is 0·8 per cent.; later in the season, in late senescence, it rose to 3·0 per cent.

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[1] Hackney, 1946.

I/N RATIOS

On the assumption that aerobic CO_2-production is represented by

$$C_6H_{12}O_6+6O_2 \longrightarrow 6H_2O+6CO_2$$

and anaerobic by

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH+2CO_2$$

the ratio of the anaerobic rate, I, to the aerobic, N, might be

$$I/N = 1/3.$$

If this value were found experimentally, it might be taken to indicate that the equations were being obeyed without complications. On this basis, numerous measurements were made during the eighteen-eighties and later,[1] and in the end it was clear that the ratio, as determined, varied within wide limits, $<0{\cdot}1$ to $>1{\cdot}0$.

A simple expectation that it will approximate to a third is based on three tacit assumptions. In the first place, it supposes that the relationship can be expressed by a simple ratio, or in other words that the two respiration rates do not drift, or drift along parallel paths; since otherwise the ratio must necessarily vary with time. Secondly, it assumes that the rate in air (20·6 per cent. O_2) is the only aerobic rate since, if aerobic CO_2-production varies with oxygen concentration, the I/N ratio will vary too. Finally, it was assumed that sugar continues to be consumed at the same rate both in air and in nitrogen. It is now clear that none of these assumptions is valid. Having reviewed all the results then available, Kostychev commented that it was doubtful whether any theoretical meaning could be attached to them because 'the rate of anaerobic respiration generally decreases from hour to hour'. Examination of Fig. 50 shows that it may increase as well, and that a significant I/N ratio can only be selected, if at all, by inspection of more or less prolonged drift curves. In Fig. 50 the aerobic and anaerobic curves eventually take up parallel courses, and it was the ratio of these adjusted rates that was used on p. 154. In 1928 F. F. Blackman proposed another method of making the comparison, based on Parija's experiments with apples. When apples were moved from air into nitrogen, the CO_2-emission followed a complex drift, which varied to some extent with the age and variety of the apple. A typical transition is diagrammatically illustrated in Fig. 54. The visible effect of the removal into nitrogen is a rapid increase of CO_2-output which, having attained a maximum, more slowly declines. It may be supposed that the deprivation of oxygen alters the rate of CO_2-production at the respiratory centres more or less immediately. The rate and the internal concentration of CO_2 both rise, some of the first-formed CO_2 being retained in solution in the tissues. For this reason the CO_2-emission rate measured externally does not leap instantly to the true rate of CO_2-production at the respiratory centres; but exhibits a lag lasting over several hours. The correctness of this interpretation of the transition has been further supported by the experiments of Watson; and Boswell and Whiting (1940) have directly measured the retention of additional dissolved CO_2 in potato tissues after such transitions. After the maximum of the nitrogen curve is reached, the emission rate reflects the true rate of CO_2-production. Blackman therefore proposed that the anaerobic rate corresponding with the final rate in air could be estimated by extrapolating the descending curve back to the moment of transition. The value for the anaerobic rate thus arrived at is shown in Fig. 54 by a circle. On removal of the fruit from nitrogen to air, a converse series of changes occurs and the aerobic component of the ratio may be estimated by a similar extrapolation. The air value thus obtained is also shown in Fig. 54 as a circle, and may be used for comparison with the final anaerobic rate. Good agreement was obtained in the ratios derived from the two directions of transition. A valid method of comparing aerobic and anaerobic CO_2-production is thus arrived at, and has been the means of achieving important results.

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[1] Wilson, 1882; Moeller, 1884. See Kostychev, 1927, for review of results to 1924. The symbols are the initials of intramolecular (now anaerobic) and normal respirations respectively.

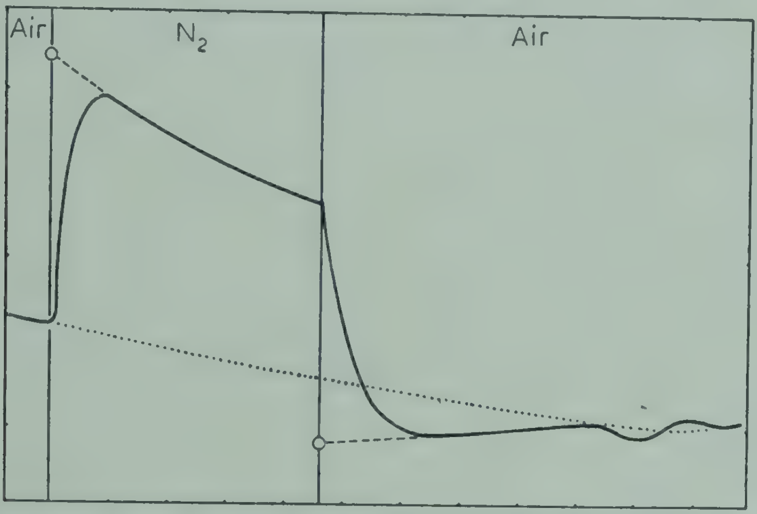

FIG. 54. Diagram to illustrate Blackman's method of comparing aerobic and anaerobic rates of CO_2-emission. Initial rates obtained by extrapolation are shown as circles. The dotted line shows the course of the CO_2-drift to be expected if the apples had remained continuously in air.

It does not, however, provide an *I/N* ratio that can be used to test the respiration equations. Reference to Figs. 51 and 52 will show that aerobic CO_2-production rates vary considerably at oxygen concentrations around atmospheric, and that a variety of ratios could be obtained according to taste by varying the oxygen concentration. In this connexion the atmospheric pressure of oxygen is a chance value within the range and of no special significance. At lower oxygen concentrations the CO_2-emission would be of mixed origin, so at what concentration should the comparison be made? Forward has suggested that the correct value for comparisons of this sort is the critical concentration of

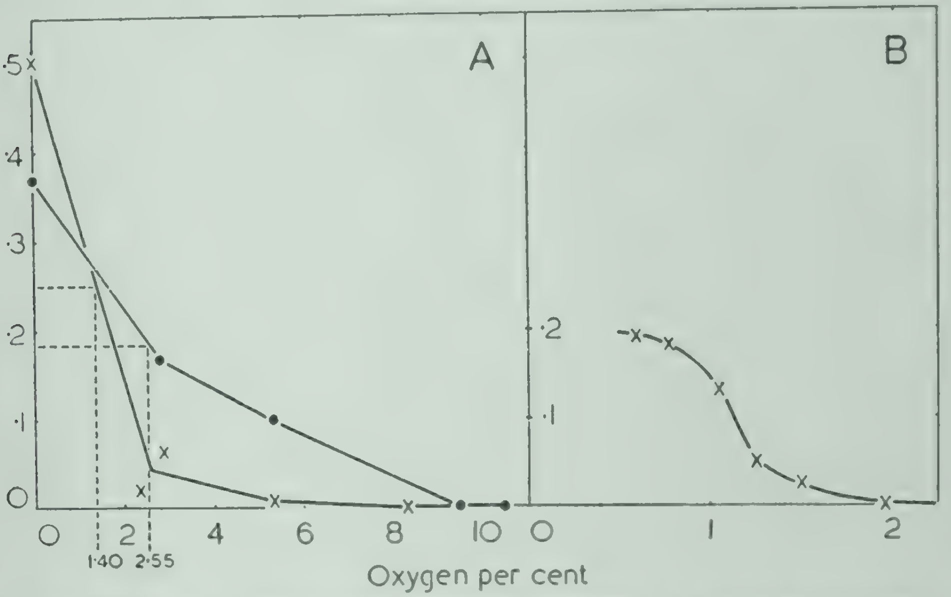

Fig. 55. Extinction of anaerobic respiration by oxygen. A. Alcohol production by senescent Bramley's Seedling apples when green-yellow to yellow. Ordinates represent increase in percentage alcohol number (alcohol + acetaldehyde) per 100 hours. ●, 1930–1 season; ×, 1931–2 season. After Thomas and Fidler. Broken lines added to show extinction coefficients at 2·55 and 1·40 per cent. respectively. B. Anaerobic CO_2-production by barley seedlings. CO_2 in air = 1·0. After Forward.

oxygen at which carbon expenditure is at a minimum. This corresponds, according to her interpretation of the data, both with the point of inflexion of the CO_2-emission curve and the extinction point of anaerobic respiration. If the last could be determined, it would be a logical choice; but we have already seen that in apples and cherry-laurel leaves there is no finite extinction point, but a continuously diminishing fade-out as oxygen concentration increases. This is also evident in Forward's own barley data (Fig. 55B), though the characteristics of the material precluded the degree of accuracy possible with apples. We must regretfully conclude that no single I/N ratio can be used to decide whether a tissue passes simply from oxidation of sugar to an alcoholic fermentation on being moved from air to an atmosphere of nitrogen.

If one is convinced that hexose oxidation and breakdown according to the two equations do occur in plant tissues, then the determination of *I/N* ratios in circumstances that ensure equal rates of hexose consumption have this importance—any deviation of the ratio from 0·33 will imply that the story is incomplete and that other processes are occurring simultaneously. The method will be valid at any concentration of oxygen where anaerobic CO_2-production is negligible. These postulates were accepted by F. F. Blackman in his analysis of a detailed series of results with Bramley's Seedling apples.[1] By the method of extrapolation illustrated in Fig. 54 ratios were obtained from estimates of the CO_2-production immediately before and immediately after the fruit was moved from one atmosphere to the other. In a number of moves between air and nitrogen the values of the ratio were found to cluster round 1·5 and 1·33; in short, CO_2-production rose considerably instead of dropping when oxygen was removed.

Blackman's assessment of glycolysis rates

Assuming that the anaerobic equation accounts for all the CO_2-production, one may calculate Gl, the rate of glycolysis or hexose breakdown, from the rate of CO_2-emission, NR, so long as oxygen is excluded.[2] Counting in C-atoms, Gl = 3NR.

To obtain a measure of glycolysis rate in the presence of oxygen, Blackman assumed that the final rate in air was the same as the initial rate in nitrogen. There is no *a priori* reason why the enzymatically catalysed rate of glycolysis should change more slowly than the enzymatically catalysed rate of CO_2-production. Forward, working in Blackman's laboratory, found reason to suppose that in barley seedlings the change of glycolysis rate is rapid, and that this method of estimating it cannot, therefore, be applied. The evidence for its validity in apples is circumstantial and derived from the fact that, when a change was made from air to 100 per cent. external oxygen supply, the change to the new rate of CO_2-emission was slow, lasting for as much as 45 hours. A similar protracted fall of about the same duration was observed in stepping down from air to 5 per cent. oxygen. Blackman found it 'hard to believe that it would take 45 hours for a pure oxygen rate to adjust itself', and regarded the change as due to an altered carbohydrate balance. He commented: 'The form of the rising transition is exactly that of an approach to a reversible equilibrium rising at first steeply and then slower and slower till the equilibrium state is reached.' In short, he envisaged a change of glycolysis rate with changing oxygen concentration; but one which was achieved slowly and which could be ignored over very short periods of time. An interesting comparison may be made with the effects of temperature changes on carbohydrate equilibria, and the slow adjustments of respiration rate that result from these (p. 33).

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[1] Blackman and Parija, 1928; Parija, 1928; Blackman, 1928.

[2] Blackman wrote NR for CO_2-production in pure nitrogen and OR for CO_2-production in air or other oxygen mixtures because he did not wish to commit himself to the use of established terms like anaerobic and aerobic respiration.

Given, on the basis of the above considerations, that initial NR/final OR = 1·5, and Gl = 3NR, then aerobic glycolysis must equal 4·5 OR, i.e. $4\frac{1}{2}$ times as many carbon atoms are involved in the hexose fission as appear in the CO_2 released. Since no glycolytic product was known to accumulate on so large a scale, it was presumed that an oxidative re-synthesis must be in operation.

Before accepting the fact of the discrepancy, one other question calls for attention. The calculation is based on the assumption that the alcoholic fermentation equation accounts for the whole of the anaerobic CO_2-production. This is unlikely to be numerically correct. Fidler (1933*b*) has made simultaneous measurements of the CO_2-production and alcohol formation of apples, and finds that the ratio between them varies widely with season, variety, and individual sample. Estimates of the ratio C_2H_5OH/CO_2 for Bramley's Seedling varied from 0·58 to 0·97, and the ratio of the carbon equivalents of alcohol+CO_2/carbon equivalents of CO_2 varied from 2·2 to 3·0. If the fermentation equation represented the only source of CO_2, the two numbers should be 1·0 and 3·0 respectively. In view of such wide variations it is not easy to regard an alcoholic fermentation as the only possibility of CO_2-production by apples placed under nitrogen; nor is the source of the aerobic CO_2 quantitatively accounted for. Watson found that apples kept in air consistently had R.Q.s above unity, which could not be attributed to acid release of retained CO_2 nor to oxidation of malic acid. The R.Q. values usually lay between 1·1 and 1·3 and, if they had happened to result from the consumption of substances with R.Q.s not greatly different from 1·0, hexoses would have been responsible for very little of the CO_2-output. This actually seems unlikely, and the only substances known to be consumed in considerable quantity by stored apples are carbohydrates and malic acid.[1] Taking Fidler's lowest value of the alcohol/CO_2 ratio, we might suppose that only 0·58 of the anaerobic CO_2 had a carbohydrate origin, and that the glycolysis rate was about half the estimated. Glycolysis in air would then be 4·5/2, i.e. 2·25 C-atoms would be glycolysed for each one appearing as CO_2. This is a maximal estimate of error and still allows for an excess of glycolysis over CO_2-production in air.

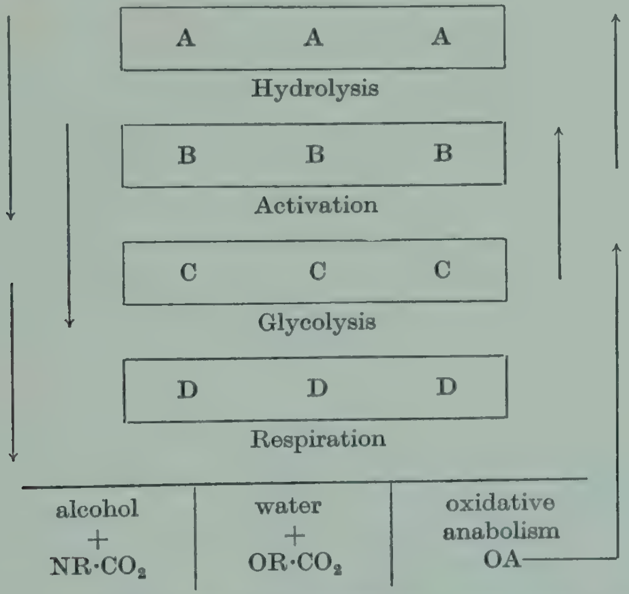
While, at the present time, it cannot be shown that the extrapolation method gives a correct quantitative measurement of glycolysis rate, the major conclusions which Blackman drew from its use

[1] See, for example, J. F. Turner, 1949.

with apples are beyond the reach of possible errors. These conclusions were not, moreover, the result of a few isolated or empirical applications of the principle, but were drawn from the prolonged contemplation of a large collection of data for a single type of material. There is nothing to indicate that the method can be more or less mechanically applied to other species and tissues. The large-scale study of barley seedlings undertaken from this viewpoint by Forward has confirmed both the general conclusions and the need for caution in applying the method.

BLACKMAN'S HYPOTHESIS

To systematize the results of his kinetic studies of apple respiration, Blackman proposed the following schema. It was explicitly a system for apples, though it took cognizance of earlier work with other materials and has since, by subsequent workers, been brought into relation with other species. In 1928 when it appeared, direct investigation of the intermediate respiratory stages in plants had hardly begun, and the scheme of fermentation outlined in Chapter VI was still in an early stage of development. The scheme envisaged a series of linked reactions divided into consecutive groups which could be recognized by kinetic characters. To avoid premature commitments, it represented intermediate substances only by a formal series of letters. At the present time it is generally recognized as the best framework for the investigation of respiratory mechanism in plants, and will therefore be detailed. The diagrammatic representation was:



In the first stage, regarded as reversible, reserve substances, A, are mobilized to free hexoses, B. The next step is an activation of hexoses into 'heterohexoses with the less stable type of internal ring structure'. The heterohexoses were regarded as the direct substrates of glycolysis. Glycolysis carried the meaning that we have adhered to in Chapter VI, i.e. the splitting of suitably prepared hexose to a series of fission products, mainly of 3-C status. The glycolysis products, collectively symbolized by *D*, led to alcohol+CO_2 under nitrogen. In the presence of oxygen, they had two alternative fates; they might be fully oxidized to CO_2+water, or they might be 'built back into the stream of metabolites', even being resynthesized to carbohydrate level. The earlier stages were held to be reversible; but the later irreversible, at least to the extent that conditions for their reversal did not occur in the natural system. The importance and novelty of this scheme lay in the attitude adopted towards oxygen, which was credited with three major respiratory roles. It was regarded as reacting directly in a specific oxidation, as inducing resynthesis, and as increasing the concentration of the substrate for glycolysis.

The reasons for these proposals and their standing in the light of some later additions to knowledge are considered in the following paragraphs; the intermediate reactions that may constitute their biochemical mechanisms are the subject of Chapters VIII and IX. The possibility of distinguishing the separate oxygen effects depended to a considerable extent on the fact that glycolysis itself could be supposed unaffected. The evidence offered was that 'oxygen has no direct effect upon the zymatic glycolysis of sugar in yeast'. The most essential aspect of the hypothesis is that the rate of formation of *D* is reversibly affected by the presence of more or less oxygen above the requirements of the terminal oxidation, and must depend on the position of equilibrium in some prior reaction. Although many glycolytic reactions can be reversed (cf. Chapter VI), the equilibrium usually lies far to the right and labile carbohydrate equilibria are much more likely than glycolytic ones to show significant fluctuations. We must therefore regard the siting of the third oxygen effect as a matter of probability rather than established fact. The evidence for its existence is much stronger, but needs a prior consideration of the characteristics of the direct oxidation.

Oxidation

Since the glycolysis products were known not to be autoxidizable, 'specific catalysts of oxidation' were presumed to compete with the anaerobic process for *D*. It is important that this oxidation was pictured as 'being of high oxygen affinity and irreversible so that the consumption of the available oxygen is complete'. This is consistent with what is known of cell oxidases (Table 29). It might be added that, if the oxidizing systems are to inhibit anaerobic respiration more or less completely, they must have a fairly high affinity for their reducing substrates as well. This also seems to occur. Table 29 collects the 'half-saturation' concentrations of the substrates of several enzymes falling into the various classes with which we are concerned, and also the corresponding value for oxygen in the respiration of a number of species.

TABLE 29

Substrate concentration for half-saturation (K_m) *of some enzymes*[1]

	Enzyme	*Substrate*	*Concentration*
Glycolysis enzymes . .	yeast zymase	glucose	6×10^{-3} M
	,, carboxylase	pyruvic acid	1×10^{-2} M
Dehydrogenases . .	cucumber malic d.	malate	7×10^{-3} M
	,, citric d.	citrate	2×10^{-5} M
	yeast lactic d.	lactate	5×10^{-3} M
Flavoprotein oxidase ..	glucose oxidase	O_2	c. 3×10^{-3} M
Oxidases: *Tenebrio* . .	mixed oxidases	O_2	$< 1{\cdot}5 \times 10^{-6}$ M
Yeast . .	,, ,,	O_2	$< 0{\cdot}5 \times 10^{-7}$ M
Micrococcus .	,, ,,	O_2	$< 1{\cdot}0 \times 10^{-8}$ M

Oxygen concentrations for 'half-saturation' of the respiration mechanism[2]

	Temperature, °C.	*Concentration*
Tubercle bacillus	37·9	$1{\cdot}1 \times 10^{-3}$ M
Rice seedlings	23·5	$> 1{\cdot}2 \times 10^{-3}$ M
Apples	22·0	$> 1{\cdot}8 \times 10^{-3}$ M
Onion roots	26·0	$5{\cdot}7 \times 10^{-3}$ M
Barley seedlings	22·5	$2{\cdot}4 \times 10^{-3}$ M
Potatoes—low sugar content . . .	..	$1{\cdot}0 \times 10^{-3}$ M
,, —high sugar content . .	..	$3{\cdot}2 \times 10^{-3}$ M
Arum flower stalk	30·0	$0{\cdot}7 \times 10^{-3}$ M
,, spadix	30·0	$> 3{\cdot}6 \times 10^{-2}$ M

Dehydrogenases are the type of enzyme by means of which the oxidation system oxidizes glycolysis products. It appears from the table that the oxidases reacting with oxygen itself have an extremely high affinity for their substrate. Further, extracted cytochrome oxidase is found to be fully saturated within the range 10^{-3}–10^{-5} atm. ($\equiv 4 \times 10^{-5}$–4×10^{-7} M) oxygen.[3] The dehydrogenases have values intermediate between those of the oxidases and carboxylase. If this can be taken as typical of the aerobic and anaerobic systems competing for the labile intermediate *D*, the oxidation system will be in a favourable position. The exceptionally high affinity of the oxidase towards oxygen may keep the dehydrogenase fully oxidized even at low oxygen concentrations. With a substrate-affinity ratio in its favour—apart from a high excess of anaerobic enzyme—it will suppress the anaerobic system more or less completely. This accords with observation. The situation might be rather different in a tissue depending on a flavoprotein oxidase with K_m of the order 10^{-3} M.

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[1] Cited from Haldane, 1930. Glucose oxidase from data of Laser, 1952.

[2] Calculated from data of various authors previously cited.

[3] Warburg and Kubowitz, 1929.

Oxidative anabolism

Blackman's second role for oxygen was the induction of resynthesis, and it is still true, as when he wrote it, that 'it is round this conception that problems are densest'. Oxidative anabolism is still not a substance nor a series of reactions; but, as far as evidence goes, only a magnitude.

Hexose activation

The third role proposed for oxygen was the activation of normal to 'hetero'-hexoses. Assuming that apple oxidases have the usual high oxygen affinity, the rate of CO_2-production in the higher oxygen concentrations cannot be supposed to depend on its direct reaction with the oxidases. The 'half-saturation' value indicated by Fig. 45 is $> 1{\cdot}8 \times 10^{-3}$ M, many thousand times greater than the oxidase values; and other tissues show similar figures (Table 29). Although Fig. 56 and the potato entries in the table show that they are related to sugar concentration, the variations are relatively small. With so much lower an affinity for oxygen, the rate-controlling process will not compete seriously with the oxidation, and this is in line with Blackman's suggestion that very low oxygen pressures would be ineffective and that the production of *D* would only be accelerated by oxygen after the oxidation system was fully saturated. This he expected to occur at about 5 per cent. oxygen. In the absence of free internal oxygen there would be a characteristic 'deoxygenate' concentration of *D*.

The suggestion that the 'more active hetero-hexoses' might prove suitable representatives of *C*, the glycolysis substrate, now probably needs modification. The hetero-hexoses, or, in modern terminology, the furanoses, possibly do not exist as free sugars even to the small extent envisaged. In their place we may put the hexose-phosphate esters, which fulfil the kinetic requirements and on other grounds are probable participants in respiration. The most likely step in phosphorylation to be accelerated by oxygen is the formation of ATP, which is also able to proceed anaerobically during fermentation. The formation of hexosemonophosphates from polysaccharides is independent of ATP, so the link in the carbohydrate chain most likely to be accelerated is the phosphorylation of fructose-6-phosphate to fructose-1, 6-diphosphate (cf. p. 116). This idea is supported by the fact that hexosemonophosphates are usually present in measurable amounts, whereas hexosediphosphate concentration is always very low (p. 111), i.e. the rate of the diphosphate formation is slow relative to the stages which both precede and follow it. If these conjectures are correct, the hydrolysis and activation stages of the schema must include phosphorolysis and phosphorylation.

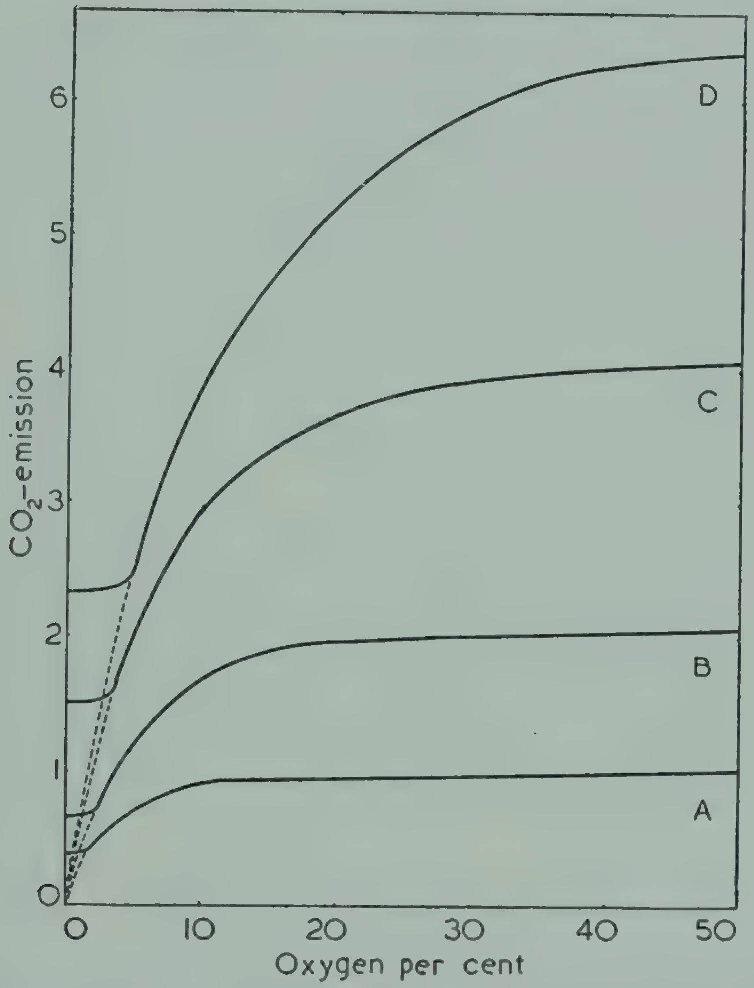

FIG. 56. Relation of CO_2-emission rate to oxygen concentration and sugar concentration in potatoes. The broken lines indicate the presumed course of aerobic CO_2-emission. A, B, C, D, curves for increasing sugar concentration. From a diagram by F. F. Blackman based on the data of J. B. Singh.

THE PASTEUR EFFECT

Pasteur[1] recorded that the alcoholic fermentation of yeast is reduced by the presence of gaseous oxygen. As criteria of the amount of

[1] *Études sur la bière.*

oxygen may keep the dehydrogenase fully oxidized even at low oxygen concentrations. With a substrate-affinity ratio in its favour—apart from a high excess of anaerobic enzyme—it will suppress the anaerobic system more or less completely. This accords with observation. The situation might be rather different in a tissue depending on a flavoprotein oxidase with K_m of the order 10^{-3} M.

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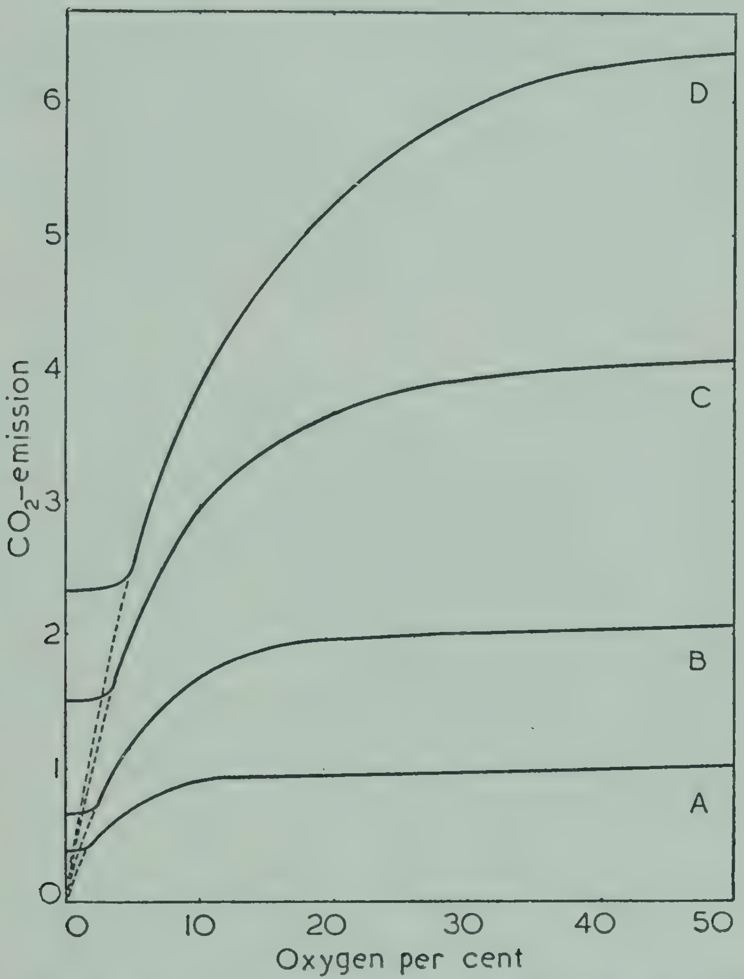

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THE PASTEUR EFFECT

Pasteur[1] recorded that the alcoholic fermentation of yeast is reduced by the presence of gaseous oxygen. As criteria of the amount of fermentation, he referred both to the amount of sugar decomposed and to the weight of yeast produced. He was not concerned with the rate of fermentation per unit time; but with the amount of fermentation per unit weight of yeast formed. Thus,

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[1] *Études sur la bière*,

> If we supply yeast with a sufficient quantity of free oxygen for the necessities of life, nutrition and respiratory combustion, it ceases to be a ferment, that is, the ratio between the weight of the plant developed and that of the sugar decomposed is similar in amount to that in the case of fungi (3 to 4). On the other hand, if we deprive the yeast of air entirely it will multiply just as if air were present, although with less activity, and under these circumstances, moreover, we shall find the greatest disproportion, all other conditions being the same, between the weight of yeast formed and the weight of sugar decomposed.[1]

In another place he refers to the disproportion between the amount of synthesis and the fermentation 'as estimated by the weight of product formed'. He also considered intermediate oxygen concentrations saying, 'if free oxygen occurs in varying quantities, the ferment-power of yeast may pass through all the degrees comprehended between the two extreme limits of which we have just spoken' (p. 259). The formation of cellular material was thus seen to replace fermentation progressively with increasing oxygen concentration. Anaerobically, the sugar of a solution would be fermented almost quantitatively to alcohol and CO_2; but aerobically three-fourths of it might become cell material. The emphasis was thus on the replacement of fermentation by synthesis. It would seem logical, therefore, to call such a change a Pasteur effect, and in botanical literature this, or something like it, is usually understood. Unfortunately, there has been no co-ordination among biologists in the use of the term which at its widest has been defined as 'oxygen inhibition of fermentable processes', whether with accompanying synthesis or not. Burk, the author of this definition, has listed 70 variants that he considers to fall within its scope. Plant respiration might be added as a seventy-first.

We shall here consider the term mainly in its implication of a switch from fermentation to synthesis and, for a start, point out that there is no implication of any necessary change in the amount or rate of sugar decomposition. On transfer from nitrogen to air, sugar decomposition may be increased or decreased or show no change; there is, for example, no contradiction or paradox in Blackman's hypothesis that the suppression of anaerobic CO_2-output from apples is accompanied by an increased rate of glycolysis. They can be two independent effects of oxygen.

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[1] p. 259, English translation.

In active young tissues, the syntheses are those of growth and are ultimately very various, involving eventually not only the carbohydrates but other metabolic fractions as well. In tissues actually photosynthesizing, it is possible that growth syntheses are independent of respiratory intermediates, but in embryos, young roots, and other dependent organs, resynthesis from glycolysis products seems probable. It might be supposed that the Pasteur effect would disappear as the tissues aged and growth slowed to a standstill. This may happen, but apparently not invariably. Blackman's oxidative anabolism would fall within the restricted limits of our definition, and was supposed to be vigorous in apples past the stage of growth syntheses. In the absence of growth or accumulation of waste or intermediate products, it can only be suggested that synthesis becomes a cyclic resynthesis of carbohydrates, an explanation first put forward by Meyerhof (1919) in connexion with yeast.

The usual method of trying to find out whether the Pasteur effect operates in higher plants has been to determine I/N; values $> 1/3$ being taken to indicate its presence. Some of the insufficiencies of this method will be apparent from the discussions on pp. 158 *et seq*. The minimum requirements are prior knowledge of the accompanying glycolysis rates, oxygen concentration curves, and proportions of CO_2 produced according to the respiration and fermentation equations respectively. It is rarely that these have been available.

Recent authors[1] have tended to emphasize a conservation of carbohydrate, or at least of carbon[2] as a feature of the Pasteur effect. Only through the idea of a resynthesis, as suggested by Meyerhof and Blackman, can this be assimilated with the original conception of Pasteur outlined above. There is now some direct evidence of carbohydrate conservation in the presence of oxygen for yeast[3] and apples;[4] but none of equal force to show that this is due to a cycle of resynthesis. Indeed, there are results with yeast[3] that may point the other way. In air or oxygen it showed a somewhat greater formation of polysaccharide and other products of growth than in nitrogen; but also a much larger residue of sugar. The sugar conserved was in the form originally provided, whether glucose or fructose. If the sugars were first broken down to glycolysis products and then resynthesized, it seems most probable that they would always reappear in the normal form, which in yeast is glucose. It seems improbable that glucose should be resynthesized as glucose and fructose as fructose. On these grounds Gottschalk has suggested that aerobic sugar conservation with yeast is not due to resynthesis, as supposed by Meyerhof, but to the reduction by some means or other of actual breakdown.

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[1] K. C. Dixon, 1937; Goddard and Meeuse, 1950.

[2] Turner, 1951.

[3] Gottschalk, 1942.

[4] Fidler, 1948.

Even if the idea of sugar resynthesis is to be abandoned for yeast, it does not necessarily become untenable for higher plants. They appear to be much more dependent on oxygen for growth and synthesis, and, with the exception of very young rice seedlings (p. 139), are probably incapable of either in its entire absence, whereas yeast does grow and form polysaccharide to some extent. Owing to the facility with which the higher plants inter-convert the sugars, the method of Gottschalk does not seem applicable to them, and direct evidence of resynthesis is still to seek.

THE COMMON-PATH THEORY

Most constructive work on the theory of plant respiration has been based on the idea of its close connexion with alcoholic fermentation. Curiously enough, the idea was first put forward in 1875 by an animal physiologist, Pflüger,[1] who noticed that some animal tissues continued to give off CO_2 in the absence of oxygen. He concluded that their respiration included two stages; in the first the respiratory substrates were converted to easily oxidized substances with some release of CO_2; in the second the labile intermediates were completely oxidized to CO_2 and water. The first step he called intramolecular respiration. In supposing that CO_2 is a normal product of animal anaerobiosis, he turns out to have been wrong; but in suggesting an initial stage independent of oxygen he started a most fertile idea. It was imported into plant physiology by Pfeffer[2] (1878) in the somewhat guarded words 'it is, in fact, possible that, even in the presence of oxygen, intramolecular respiration will run through as far as the formation of alcohol; or, in general, as far as the formation of end products, which will then be further oxidised as they arise'. He described this as the simplest way to account for the obvious 'genetic relationship' between CO_2-production in the presence and absence of oxygen. He does not seem to have been very firmly attached to the notion, and by 1897 recanted so far as to write: 'It is therefore possible that the formation of alcohol in the intramolecular respiration of aerobes may arise from a series of processes which are quite foreign to the normal respiratory activity, and indeed various facts point to this conclusion.'[3] The various facts were derived from experiments, carried out by Wilson[4] and Diakonov[5] in Pfeffer's laboratory, which do not now seem so compelling.

The most durable objection to Pfeffer's theory was that the higher

[1] Pflüger, 1875.

[2] Pfeffer, 1878.

[3] *Pflanzenphysiologie*, vol. i, p. 547, English translation.

[4] Wilson, 1882.

[5] Diakonov, 1886, summarized and discussed by Kostychev, 1927.

plants have very limited powers of oxidizing alcohol. This was demonstrated by Kostychev using a variety of materials,[1] and more recently Phillips has shown that alcohol, formed anaerobically by young barley seedlings, is not oxidized on return to air. Very young rice seedlings, which are exceptional in being able to germinate under nitrogen, she found able to oxidize alcohol; but older seedlings had already lost the power. On the other hand, a fairly rapid oxidation of alcohol by carrot tissues has been reported.[2] Carrot slices weighing 100 gm. placed in 5 per cent. alcohol oxidized 160 mg. per hr. at 37° C. The formation or non-formation of alcohol seems now a matter of minor importance, since, in the presence of oxygen, it must both come from acetaldehyde (p. 137) and be oxidized to it, that is to say it is necessarily a side product, not an intermediate one. If the oxidizing system becomes overloaded with acetaldehyde, or an acetaldehyde precursor, alcohol will appear in the tissues; if the supply of substrate is more moderate, oxidation will be complete at the earlier stages. This situation has been experimentally demonstrated in apples and oranges in dilute acetaldehyde vapours.[3]

The suggestion that oxidation might intervene at an intermediate stage and anticipate the formation of alcohol is associated with the names of Palladin and Kostychev, who modified Pfeffer's theory accordingly. This removed the most serious difficulty while retaining the idea of an initial reaction-path common to both aerobic and anaerobic respirations. Blackman's hypothesis is built upon the same foundation, and to Kostychev's formulation adds the conception of oxygen-induced resynthesis. This Pfeffer-Kostychev-Blackman theory has been the thread on which has hung historical continuity and metabolic integration in the study of plant respiration.

Various types of evidence have been brought forward in support of the common-path theory at different stages of its development. Those which still seem worthy of attention follow in brief:

1. The anaerobic production of carbon dioxide is universal among plants, that is to say it is coextensive with the aerobic process. *Elodea* has been reported a doubtful exception.[4]

2. After a brief period of anaerobiosis, not long enough to entail damage, many plant tissues show a temporary increase of CO_2-production when brought back into air.[5] The increase occurs rapidly and may

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[1] Kostychev, 1927.

[2] Wetzel, 1933.

[3] Trout and Kidd, 1931; Fidler, 1935, 1936, 1937.

[4] Lyon, 1923.

[5] Palladin, 1904 (*Chlorothecium saccharophilum*, a unicellular alga); Saran, 1938 (*Aralia* sp.); Singh, Seshagiri, and Gupta, 1937 (mango and guava); Gustafson, 1930 (tomatoes); Leach and Dent, 1934 (fatty seeds); Leach, 1936 (starchy seeds); Wood, Mercer, and Pedlow, 1944 (leaves of Kikuyu grass); Stiles and Dent, 1947 (slices of potato); Laing, 1940 (*Scirpus validus*).

be large, suggesting the oxidation of a product of the anaerobiosis. Parija[1] examined the drift curves of detached cherry-laurel leaves, transferred during phase 2 (p. 40) from air to nitrogen. As with apples, the rate of CO_2-production went above the air-line. A constant rate of CO_2-emission was maintained for about 30 hours, after which a steady decline to zero set in. If the leaves were returned to oxygen before the decline was far advanced, a still further rise of CO_2-emission rate took place, which was maintained for some considerable time and then returned to the normal air-line. The magnitude of the after effect depended upon the duration of the nitrogen experience. The additional CO_2 of the after effect could be added up, and in a series of experiments was found to be proportional and approximately equal to the anaerobic CO_2.[2] This was a particularly large amount and, unless the substrate of the after effect was alcohol itself, the anaerobic respiration of cherry-laurel leaves must depart a long way from the fermentation equation. Young barley leaves form little or no alcohol under nitrogen; but on return to air show enhanced oxygen uptake.[3] We may suppose that the oxidizable substance that accumulates may be an intermediate or side product of alcohol formation; but in no instance has it yet been identified. Apples which certainly form alcohol, sometimes in almost theoretical amounts, do not show augmented CO_2-production on return to air (Fig. 54, p. 159) and are unable to oxidize the alcohol.

3. Variations of conditions which affect anaerobic respiration affect aerobic respiration to the same extent. The best example of this is the effect of temperature. It has been shown for a variety of seedlings that the Q_{10}'s of aerobic and anaerobic respiration are identical and of the order to be expected of a thermochemical reaction (p. 31). This might result if a partial reaction in a common anaerobic stage were controlling the rate of both. Considerations already discussed (p. 166) make it probable that CO_2-emission rates are controlled by some reaction other than a direct oxidation, even in the presence of abundant oxygen.

It is possible to change aerobic rates of CO_2-output by means that do not affect the anaerobic. Thus it has been shown that mechanical manipulation, such as handling, of cherry-laurel leaves causes a strongly marked temporary increase of CO_2-output in air.[4] The same effect is reported for a considerable number of other leaves,[5] potatoes,[6] and beetroot slices.[7] It is observable in diminishing degrees in concentrations of oxygen down to 2·5 per cent.; but is entirely absent under nitrogen.[8] It is therefore closely bound to the aerobic phase of respiration, which must follow the anaerobic. This argument would prove double-edged if other conditions were found that would change anaerobic CO_2-output without altering the aerobic. Up to the present none have been reported.

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[1] Unpublished.

[2] F. F. Blackman, lectures in the Cambridge Botany School. See also Audus, 1940. Similar results with Shasta daisy leaves by James and Ritchie, unpublished.

[3] Phillips, 1947.

[4] Audus, 1935; Godwin, 1935.

[5] Audus, 1939.

[6] Barker, 1935.

[7] Stiles and Dent, 1947.

[8] Audus, 1940.

4. Specific inhibitors require separate consideration. A number are known which will inactivate oxidases without having any similar effect on glycolysing enzymes. The most used and studied is HCN. This actually has two effects; at high dilution, M/500 or less, it reacts with metals such as iron and copper, thus inactivating the oxidases depending on them; in more concentrated solutions, M/100 and higher, its formation of cyanhydrins with aldehydes and ketones becomes sufficiently pronounced for it to act as a substrate fixative as well. The reaction with metals is readily reversible, but the cyanhydrins are stable at ordinary temperatures at neutral or basic reactions. The usual effect of adding M/1,000 cyanide to plant tissue respiring in air is to reduce oxygen uptake greatly, CO_2-output being less affected, so that the R.Q. rises,[1] and alcohol may accumulate in the tissues.[2] Direct comparison of the effects of M/500 cyanide on young barley leaves in air and nitrogen[3] showed that, while the usual result was observable in air, there was no effect under nitrogen unless the leaf sugars were depleted. The aerobic inhibition was reversible, disappearing rapidly when cyanide was no longer supplied. Oxygen uptake and CO_2-output did not merely return to their normal level, but, for a time, showed a rise above it (Fig. 57), thus simulating the behaviour after a short period in nitrogen. At M/50 cyanide the suppression of CO_2-output was total, so that it must have included the anaerobic stage as well, and was not reversed when the cyanide was removed. This could be accounted for by supposing that it was blocking respiration in the anaerobic stage by forming a cyanhydrin with phosphoglyceraldehyde.[4] All these observations are consistent with the idea of an anaerobic stage, followed by an aerobic one.

The converse experiment of blocking anaerobic respiration should be even more illuminating. The most used inhibitor has been M/1,000 iodoacetate which inhibits the phosphorylation of hexoses, and the enzymes triosephosphate and alcohol dehydrogenases (p. 132); but has little effect on glucose dehydrogenase[5] or the terminal oxidases.[6] The last serious attempt to prove the independence of aerobic respiration from anaerobic derived its evidence from the use of this inhibitor. Following experiments on yeast,[1] Boysen-Jensen found that soaking the cotyledons of peas in iodoacetate solutions reduced their CO_2 emission rate much more in hydrogen than in air. A considerable controversy developed round the more detailed yeast results,[2] but so far as the higher plants are concerned the matter seems fairly simple. Boysen-Jensen's experiments were done before the importance of pH in controlling the rate of entry of weak acids into plant tissues was understood, and it is possible that, were they repeated with adequate experimental control, different results would be achieved. It was, however, known that iodoacetate only enters intact cells comparatively slowly, and Turner,[3] working with carrot disks, showed that the apparent failure of iodoacetate to inhibit aerobic CO_2 production was only temporary. Inhibition of yeast fermentation by M/1,000 iodoacetate was complete within an hour; with carrots under nitrogen 9 hours were needed

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[1] Thomas and Fidler, 1941*a* and *b* (apples); Genevois, 1927 (sweet pea seedlings); Genevois, 1929*b* (*Chlorella*); Machlis, 1944*a* (barley roots); Boswell, 1950 (slices of swedes, potatoes, and carrots); Marsh and Goddard, 1936*b* (carrots).

[2] Thomas and Fidler, 1941*a*.

[3] James and Hora, 1940.

[4] Cf. James, Heard, and James, p. 133.

[5] Müller, 1928, 1929, 1936; Ogura, 1939.

[6] See James, 1952, for references and details.

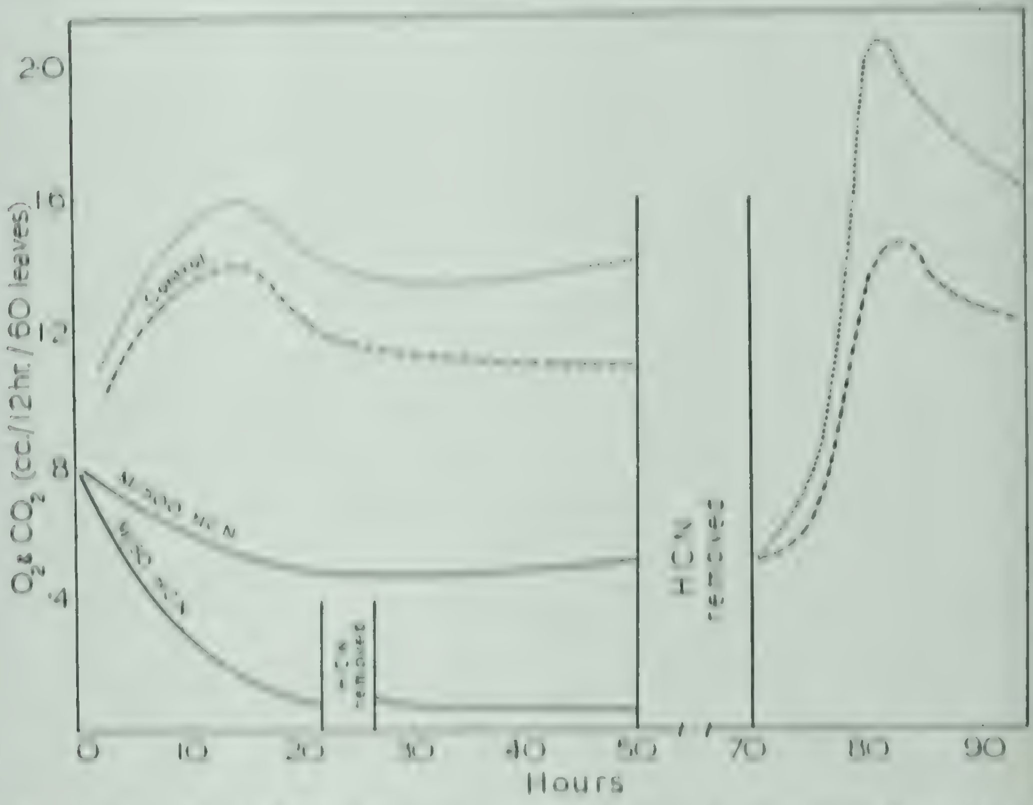

FIG. 57. The effect of HCN on the gas exchange of young barley leaves. Dotted lines oxygen, broken lines CO_2, continuous lines oxygen and CO_2 coincident. There is a break in the time scale between 50 and 70 hours. Constructed from the data of James and Hora.

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[1] Lundegårdh, 1930; Boysen-Jensen, 1931.

[2] Reviewed by Turner, 1937.

[3] Turner, 1938b.

Inhibition in air developed equally massively but still more slowly. It is now also known that, although iodoacetate does not attack the oxidases themselves, it does also reduce the rate of oxygen uptake in aerobic respiration.[1] This is also most readily explained by supposing it inhibits an earlier stage supplying oxidizable intermediates.

Iodoacetate was not, as it turns out, a very fortunate choice. It is not a highly specific inhibitor, reacting generally with thiols (—SH compounds).

$$R\cdot SH + CH_2ICOOH \longrightarrow HI + R\cdot SCH_2COOH.$$

Many enzymes contain thiol groups and are inactivated to varying degrees by iodoacetate. Singer (1945) lists 33, including several dehydrogenases which could conceivably form the proximal ends of oxidation chains where glycolysis and oxidation meet. They appear, however, to require concentrations considerably in excess of M/1,000 for appreciable inactivation.[2]

Confirmatory results have, moreover, been obtained with sodium fluoride which most actively inhibits the glycolysis enzyme, enolase. If the interpretation is right, it should be possible to annul iodoacetate and fluoride poisoning by supplying a glycolysis product for the oxidation systems to work on. The most suitable is pyruvic acid,[3] which has been shown to be formed by the glycolysing system of barley and also during its respiration (p. 134). Addition of M/40 NaF has been shown to prevent its formation.[4]

Oxygen uptake by barley roots is almost completely inhibited by 4×10^{-5} M iodoacetate at pH 4·5 in about 4 hours. Fluoride inhibition is also well marked and, with both, the full uninhibited rate of O_2-absorption can be restored by suitable additions of pyruvic acid.[5] Pyruvate relief of iodoacetate and fluoride poisoning has also been observed in spinach leaves[6] and swedes.[7]

5. Enzymes responsible for glycolysis have been obtained from numerous plant tissues. This at first took the form of showing that 'zymase', the collection of enzymes responsible for alcoholic fermentation, was active in dead preparations.[8] By degrees many of the constituent enzymes such as zymohexase, carboxylase, alcohol dehydrogenase, and also Coenzyme I and cocarboxylase have been extracted

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[1] e.g. Caldwell and Meiklejohn, 1937*b* (tomato stems); Machlis, 1944*a* (barley roots); Bonner and Wildman, 1946 (spinach leaves); Bonner, 1948 (oat coleoptiles); Boswell, 1950 (swedes); James and Beevers, 1950 (*Arum* spadix); Laties, 1949*b* (barley roots).

[2] e.g. succinic dehydrogenase, Hopkins and Morgan, 1938.

[3] e.g. Caldwell and Meiklejohn, 1937*b* (tomato stems); Bonner and Wildman, 1946 (spinach leaves); Bonner, 1948 (oat coleoptiles); Boswell, 1950 (swedes); James and Beevers, 1950 (*Arum* spadix); Laties, 1949*b* (barley roots).

[4] James, James, and Bunting, 1941.

[5] Laties, 1949*b*.

[6] Bonner and Wildman, 1946.

[7] Boswell, 1950.

[8] Kostychev, 1927, p. 97.

and recognized (p. 133). These enzymes are not inactivated by the presence of oxygen, but it has not been satisfactorily proved that they are not reversibly inhibited by the cell oxidizing systems. Thiol enzymes can be reversibly oxidized and inhibited, for example, by oxidized glutathione.[1]

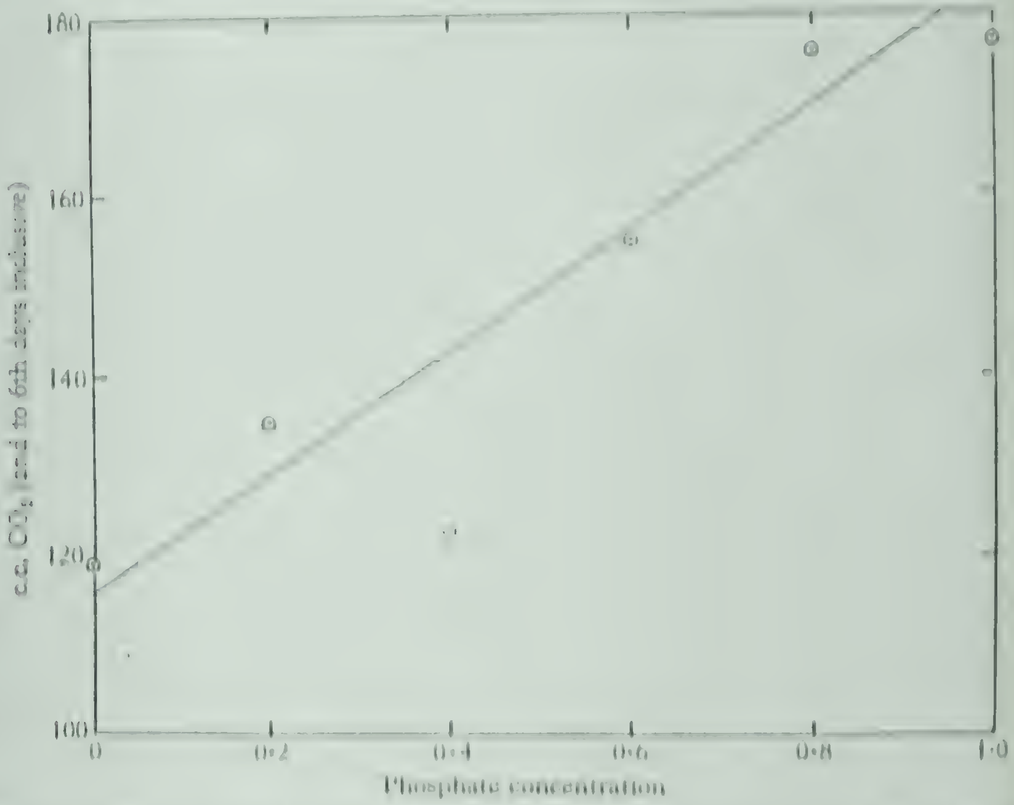

Fig. 58. CO_2-output by 80 excised barley embryos during the second to sixth days of germination. Unit phosphate concentration = 0·25 gm. $CaH_4(PO_4)_2H_2O$ per litre. The medium contained 4 per cent. sucrose. James and Arney, 1939.

By contrast with the wealth of enzymes known to affect products of glycolysis, there is no report of a hexose dehydrogenase, or other hexose-decomposing enzyme, from any higher plant. Even among the mould fungi, where a glucose dehydrogenase occurs,[2] it may be exceptional rather than the rule.[3]

6. Phosphorylation, known to be an essential preliminary to alcoholic fermentation, is also part of aerobic respiration. The aerobic CO_2 output of acutely phosphate-starved barley embryos shows a linear relation with phosphate supply (Fig. 58), and batches of normal seedlings show positive correlation between rate of CO_2 output and amount

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[1] Hopkins and Morgan, 1938.
[2] Muller, 1930; Ogura, 1930; Coulthard *et al.*, 1945.
[3] Suthers and Walker, 1932.

of esterified phosphate (Fig. 59).[1] Phosphate supply may also govern the respiration rate in phosphate-starved barley leaves.[2]

The case for the common path may thus be summarized: the capacity for anaerobic respiration is as universal as for aerobic, and an accumulation of materials results that the plant may be able to oxidize on return to air. Variations of temperature affect both processes identically; no controlling condition is known to affect anaerobic respiration without affecting aerobic respiration also. The final stages of respiration can be chemically inhibited without paralysing glycolysis; but glycolysis in air cannot be inhibited without a similar effect on oxidation; the provision of a glycolytic product re-establishes the missing oxidation. Glycolysing enzymes and enzymes capable of oxidizing glycolysis

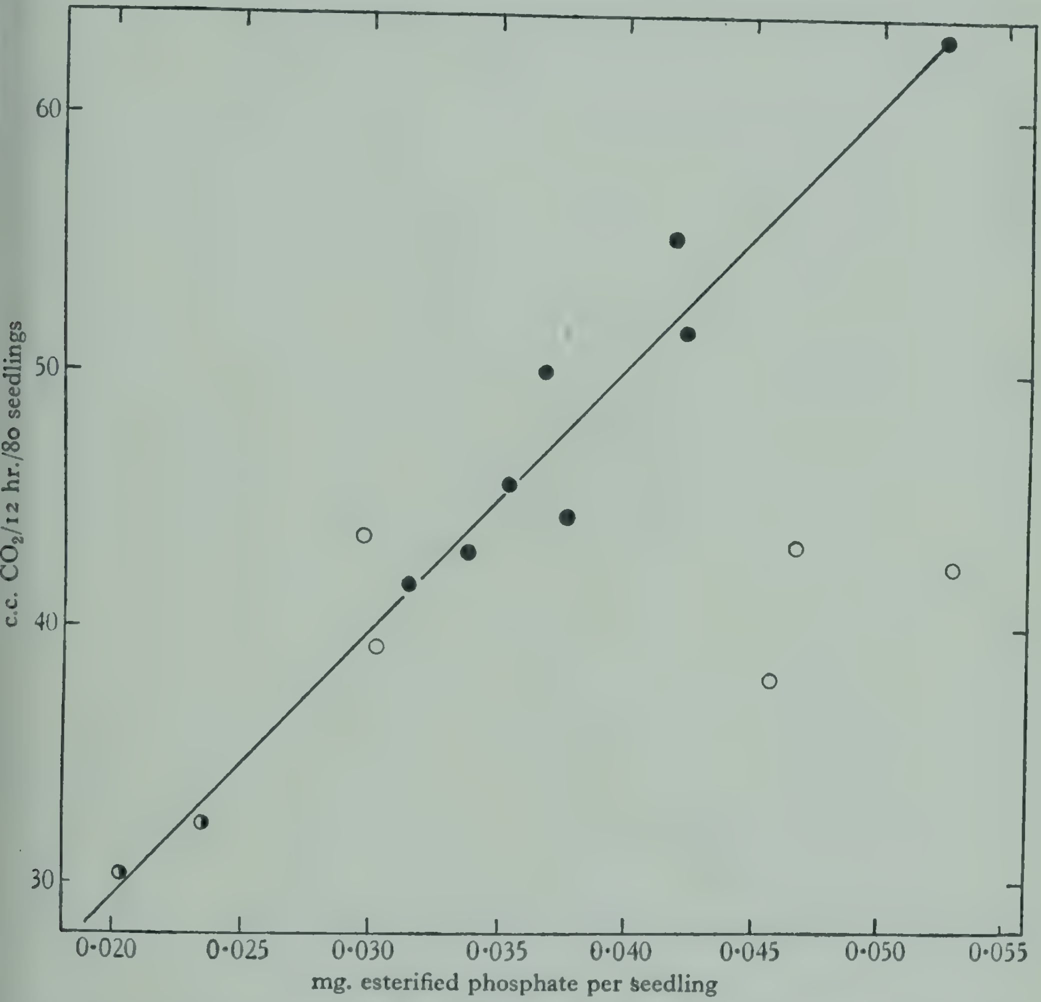

Fig. 59. The relation between the CO_2-emission and esterified phosphate content of barley seedlings. ◑, 3-day seedlings; ●, 5-day seedlings; ○, 10-day seedlings. James and Arney, 1939.

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[1] James and Arney, 1939.

[2] Said and Sankaran in Richards, 1938.

products are readily obtainable from plants; but no enzyme capable of oxidizing or decomposing a hexose sugar. Phosphorylation, the preliminary to hexose fermentation, occurs during aerobic respiration also.

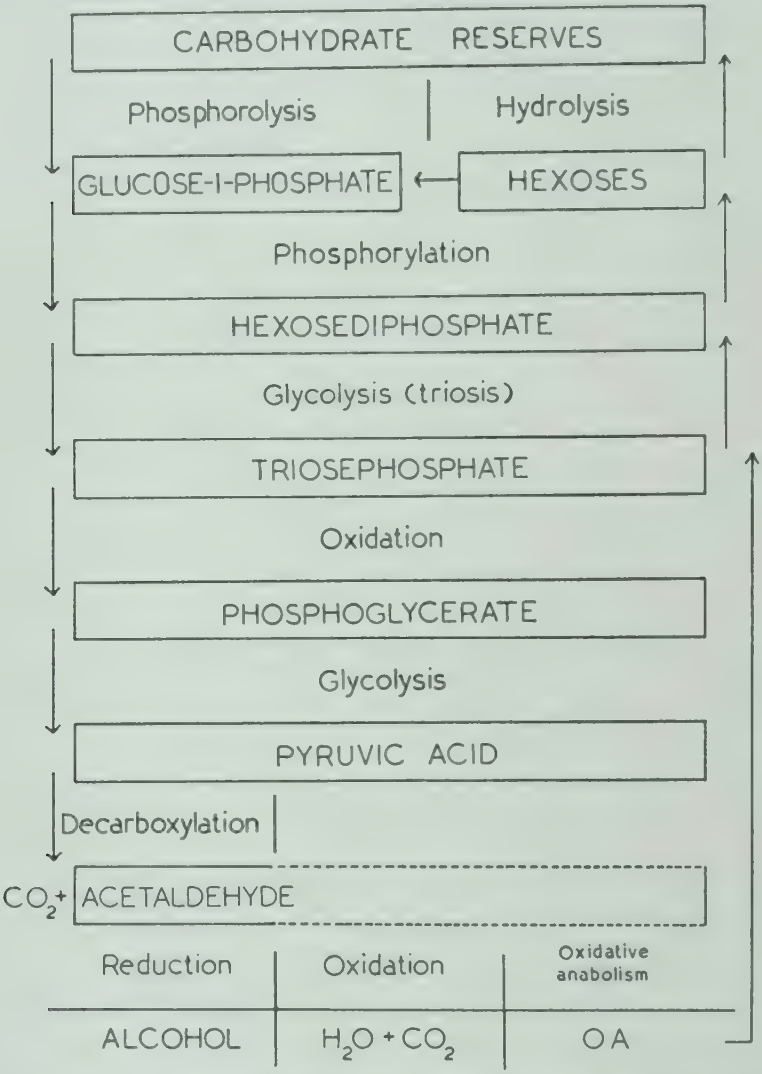
CARBOHYDRATE RESERVES
Phosphorolysis
Hydrolysis
GLUCOSE-I-PHOSPHATE
HEXOSES
Phosphorylation
HEXOSEDIPHOSPHATE
Glycolysis (triosis)
TRIOSEPHOSPHATE
Oxidation
PHOSPHOGLYCERATE
Glycolysis
PYRUVIC ACID
Decarboxylation
CO2+
ACETALDEHYDE
Reduction
Oxidation
Oxidative anabolism
ALCOHOL
H2O + CO2
OA

FIG. 60. Summary of the common-path theory of plant respiration.

Based on such evidence, the theory of the common initial path for aerobic and anaerobic respirations at present holds the field. Its existing form has developed logically from the tentative suggestion of Pfeffer through the progressive stages associated especially with the names of Palladin, Kostychev, and Blackman. In recognition of this continuity one may attempt to summarize present knowledge of the respiratory carbon flux in a development of the Blackman schema on p. 163, and in accordance with the comments following it. Fig. 60 is a summary along such lines.

VIII

OXIDATION MECHANISMS

The previous chapters have been concerned with respiration as a carbon flux; equally important is the fate of the associated hydrogen. Comparison of the two sides of the summary equation

$$C_6H_{12}O_6+6\,O_2 \longrightarrow 6\,CO_2+6\,H_2O$$

makes it appear that molecular oxygen is equally divided between carbon and hydrogen to give CO_2 and water. This does not, in fact, happen and no mechanism of biological oxidation is known that would promote such a reaction.

Palladin[1] appears to have been the first to state a possible alternative, which carries the essence of present theory. He suggested that the process is catalysed by water, and that hydrogen, derived partly from the original molecule, partly from water of its hydration, is transferred to molecular oxygen. He formulated his reactions in the equations:

$$\begin{aligned} C_6H_{12}O_6+6\,H_2O &= 6\,CO_2+(12\,H_2) \\ (12\,H_2)+12\,R &= 12\,RH_2 \\ 12\,RH_2+6\,O_2 &= 12\,H_2O+12\,R. \end{aligned}$$

It is thus indicated that all the free oxygen consumed is reduced by hydrogen, which is transferred to it through the reversible reduction and oxidation of R. R stands for a class of substances called by Palladin chromogens in the reduced state and pigments in the oxidized; they are more fully described on p. 189. Palladin (1908*a*) gave an explicit account of their role. 'In the first place these pigments do not enter directly into combination with the oxygen of the air, but through the medium of the oxidase, and secondly it is not carbohydrates that are directly oxidised by the pigments mentioned, but their decomposition products, which are produced without the intervention of molecular oxygen'; and further, 'It is obvious that pigment formations are to be taken as reversible reactions and that consequently a pigment formed in the living cell is immediately reduced again . . . '. The transfer of hydrogen from the chromogen to O_2 was catalysed by an enzyme for which the name oxidase was reserved; the H_2-transfer from the glycolysis products to the pigment was catalysed by a second enzyme, called a reductase, or in present terminology, a dehydrogenase. These could not transfer H_2 directly to oxygen; but, in addition to the natural pigments, would transfer it to a large number of artificial dyes which were bleached in the process. Air was excluded by filling the reaction tube completely with the dye solution and frozen or chloroformed tissues. On admission of air the colour returned; particularly suitable dyes were methylene blue and indigo.

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[1] Palladin, 1908*a* and *b*, 1909, 1911, 1912*a* and *b*, summary 1923; Palladin and Tolstoya, 1913.

Another important point was also stressed. 'It is noteworthy that substances whose oxidation is possible by the oxidases are never burnt to CO_2+H_2O, but only incompletely oxidised . . .'. With the further details of Palladin's theory we are not concerned; but only with its essential structure. According to the theory, respiratory CO_2 is anaerobic in the sense that it contains no oxygen derived from the air (but not in the sense that no more CO_2 is got from a molecule of sugar in the presence of free oxygen than in its absence). Free hydrogen is never released; but moves through a series of transfers from the products of glycolysis to oxygen. The stages of transfer are catalysed by appropriate enzymes. These proposals make an excellent foundation from which to start the modern study of oxidation mechanisms in plants. That is to say we need contradict none of them; but only enlarge and widen. An improved summary equation would run

$$C_6H_{12}O_6+6\ H_2O+6\ O_2 \longrightarrow 6\ CO_2+12\ H_2O.$$

OXIDO-REDUCTION

A substance is oxidized when it loses one or more electrons; the removal may be associated with four types of reaction.

1. Loss of the electron unmasks a positive charge, thus causing a change of valency

$$Fe^{++} \longrightarrow Fe^{+++}+e$$

the electron being accepted by, e.g. $\frac{1}{2}Cl_2$ which becomes Cl^-.

2. Oxygen is taken up, the electron being shared—

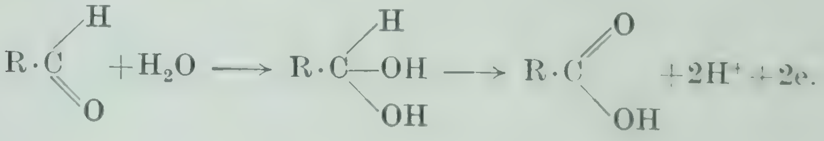
$$2\ FeO+\tfrac{1}{2}O_2 \longrightarrow Fe_2O_3.$$

3. Hydrogen is lost

$$(CH_2COOH)_2 \longrightarrow (CH\cdot COOH)_2+2H^++2e$$

the hydrions and the electrons may be accepted by the same or different receivers.

4. As 3, preceded by hydration



$$R\cdot C\begin{smallmatrix}H\\ \\ O\end{smallmatrix} + H_2O \longrightarrow R\cdot C\begin{smallmatrix}H\\ -OH\\ OH\end{smallmatrix} \longrightarrow R\cdot C\begin{smallmatrix}O\\ \\ OH\end{smallmatrix} +2H^+ +2e.$$

So far as the oxidation itself is concerned 3 and 4 are similar; but the initial hydration makes possible the oxidation of a wide range of substances not otherwise oxidizable by this means. Some glycolysis products, for example, phosphoglyceraldehyde, are among them. All the above reaction-types probably occur in plant tissues.

In seeking to modernize Palladin's theory we must divert our attention from the hydrogen flux to the flow of electrons. Glycolysis products are substances with a marked tendency to lose electrons and oxygen has a strong tendency to acquire them. Usually the glycolysis products are oxidized by reactions 3 or 4; but the hydrions and electrons of the hydrogen they lose soon part company.

The strength of the tendency for electrons to escape from a given substance can be expressed as an E.M.F. and, where the electrons will pass to an electrode, any two such tendencies can be compared in an electrolytic cell. If each half-reaction occurs in a half-cell round the electrode, electrons will flow along the connecting wire from the reaction with the greater escaping tendency towards the other, providing the circuit is completed by the migration of ions through a liquid junction. The E.M.F. may be obtained by means of a potentiometer interrupting the wire. As a standard with which to compare all other oxido-reductions, the system of which molecular hydrogen is the reductant and the hydrogen ion is the oxidant, is taken

$$\tfrac{1}{2}\,H_2 \rightleftharpoons H^+ + e$$

with hydrogen pressure = 1 atm. and hydrion concentration unity. When the hydrogen half-cell is kept under these conditions the potential E_h can be measured with varying concentrations of reductant and oxidant (e.g. Fe^{++} and Fe^{+++}) in the other half-cell. The potential follows a sigmoid curve becoming more negative with increasing reduction (Fig. 61). With oxidized and reduced forms present in equal amount, the potential has a unique value, E_0, characteristic of the system, assuming standard conditions of temperature and acidity; and electrons will flow from a system of higher negative potential to one of lower. These redox potentials are thus important to our present purpose as showing in what direction an electron flow may conceivably take place, given the suitable mediators as catalysts. Other things being equal, electrons will flow from a system of negative E_0 to one of less negative or positive E_0. In using these values to predict directions of the electron transfer, considerable caution must nevertheless be observed. Not only does the potential shift with the degree of reduction of the system; but also with temperature and acidity. For this reason E_0 values and redox potential curves are usually stated for standard conditions, viz. 30° C. and pH 7·0. These were chosen for convenience of comparison with animal tissues; and, although conditions within the cell are uncertain, lower temperature and pH, say 20° C. and pH 6·0, would probably be better approximations for plants. Little error is likely to arise from this cause. The E_0 values mostly at 30° C. and pH 7·0 of a number of systems of plant-biological importance are shown in Fig. 62.

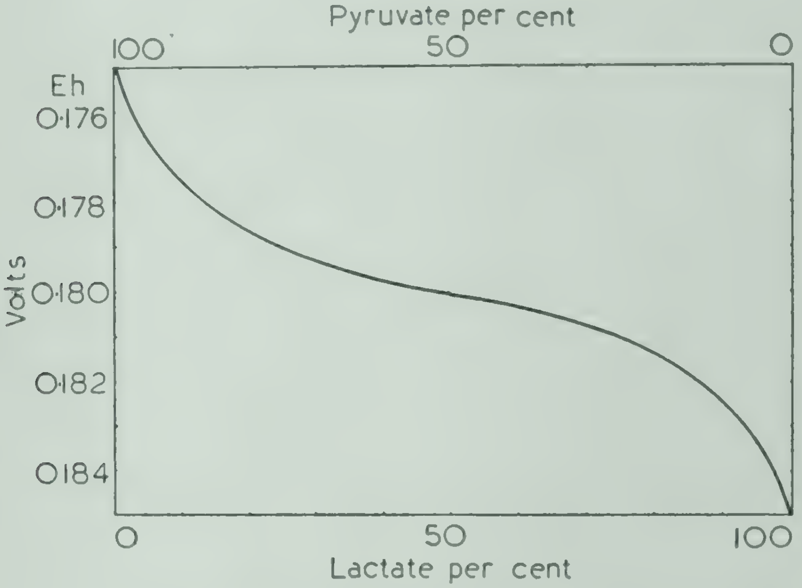


Fig. 61. Redox potential, E_h, of the system lactate $\rightleftharpoons$ pyruvate.

In general one may expect electrons to pass from the reduced form of any system to the oxidized form of any system higher in the diagram. Nevertheless, electrons will not pass direct from, say, the lactate $\rightleftharpoons$ pyruvate system to the succinate $\rightleftharpoons$ fumarate system when these and their appropriate dehydrogenases are mixed *in vitro*. To bring this about a suitable carrier must also be present; and in the artificial system a redox dye may be used. For good results it should have an E_0 between those of the two systems. Here, an appropriate choice from the range of redox dyes provided by Clark would be methyl capri blue, $E_0 = 0{\cdot}060$ V. Promoting the flow of electrons in living systems, similar natural carriers must also be present; the best known are shown on the right of Fig. 62. It is not to be assumed from the above example that, during the oxidation of lactate in living cells, electrons derived from the lactate are acquired by fumarate on their way to oxygen. Most of the reduced substances on the left of the diagram are probably better regarded as possible glycolysis products, each providing the starting point of a stream of electrons. Our present inquiry is concerned with the paths of electron flow from such substances to oxygen.

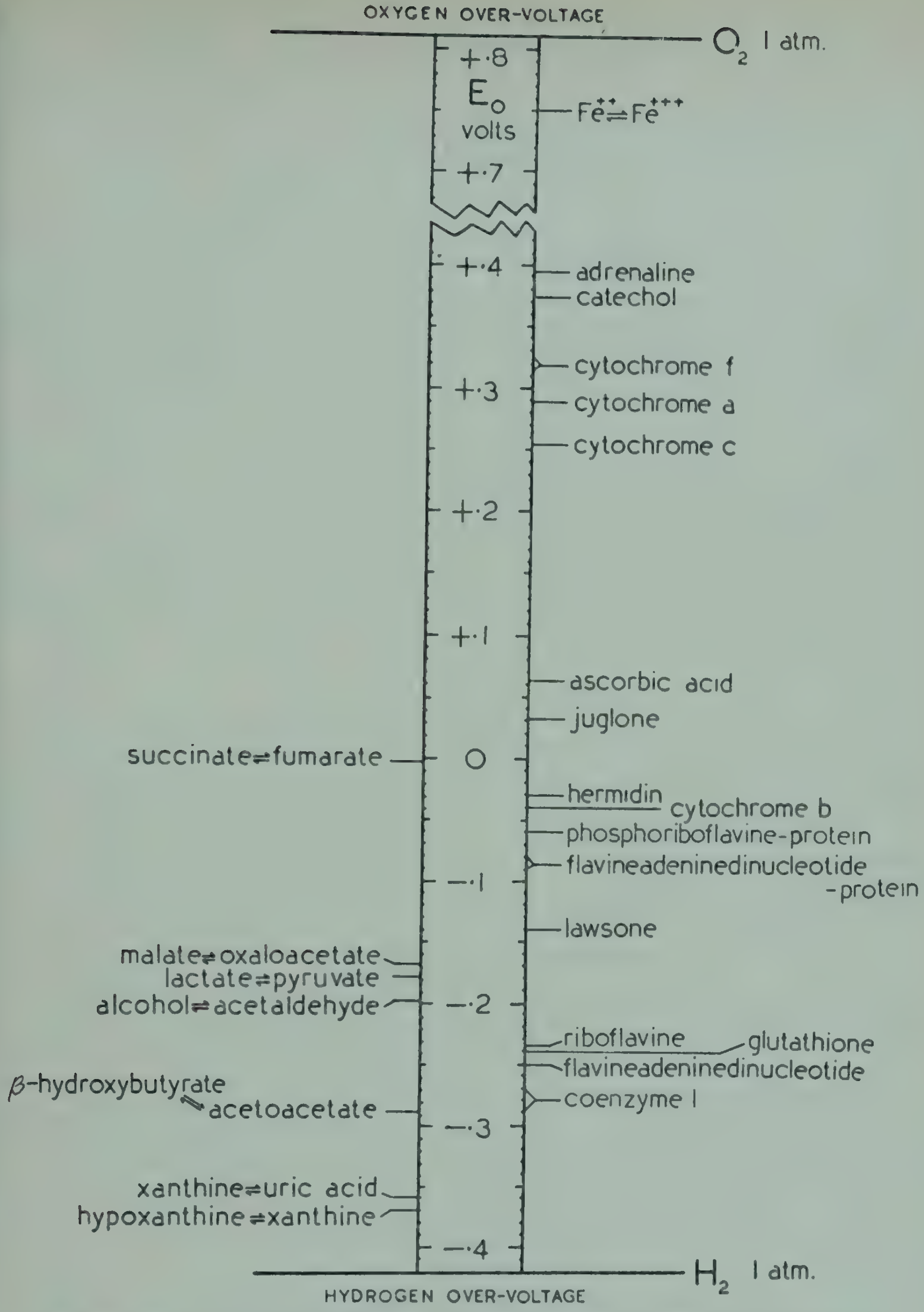
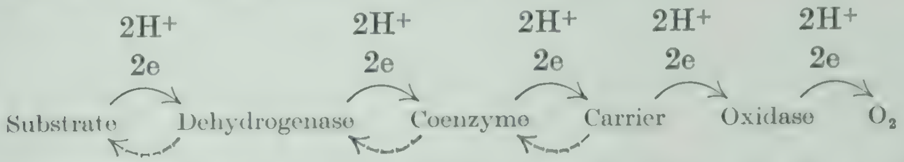


Fig. 62. Redox potentials, E_0, for a number of systems of biological interest.

For an elementary and convenient account of the principles underlying the measurement of redox potentials see Mansfield Clark, 1939. Data on which the figure is based are given by Clark ($Fe^{++} \rightleftharpoons Fe^{+++}$ and adrenaline); Ball, 1937 (ascorbic acid), 1938 (cytochromes *a*, *b*, and *c*), 1939 (flavine adenine dinucleotide and protein); Ball and Chen, 1933 (catechol); Borsook and Schott, 1931 (succinate ⇌ fumarate); Barron, 1939 (hermidin); Friedheim, 1934 (juglone and lawsone); Laki, 1937 (malate ⇌ oxaloacetate); Michaelis, Schubert, and Smythe, 1936 (riboflavin); Green and Dewan, 1937 (Coenzyme I); Green, 1934 (xanthine); Barron and Hastings, 1934 (lactate ⇌ pyruvate); Adler and Sreenivasaya, 1937 (alcohol ⇌ acetaldehyde); Green, Dewan, and Leloir, 1937 (β-hydroxybutyrate ⇌ acetoacetate).

Speaking of this flow, there has already been occasion to name a variety of agents whose relations may be summarized,



Some at least of the stages are reversible as indicated by the dotted-in arrows; but in a steady state of respiration reaction is progressing from left to right. Probably no fixed relation is maintained between electrons and hydrions, the latter being exchanged as required with the environment. Although the substrate molecules usually lose 2 electrons, each is in all probability removed as a separate act, and this is certainly true at some intermediate stages. Each substance named represents a redox pair with a characteristic E_0; when all are present, electrons will descend the staircase of diminishing electron potential and free energy so provided.

PLANT DEHYDROGENASES

A full-scale biochemical account of these and other oxidizing systems is not obligatory for our present purpose, and only those features likely to be of respiratory significance need be mentioned. This group of enzymes, whose existence was first indicated by Palladin in 1908, has been kinetically studied largely by simple improvements of his technique of allowing the dehydrogenase to reduce and so decolorize methylene blue, or other artificial redox dyes, in the absence of oxygen. Thunberg introduced a convenient exhaustion tube from which air can be removed and replaced if desired by an inert gas. With this device he examined a number of tissues, including some from plants,[1] and found that the presence of dehydrogenases was widespread. More recent investigations have shown that dehydrogenases are highly specific with regard to both hydrogen donor and acceptor. The enzyme does not in fact react directly with the artificial dye used as indicator, but only at one or two removes through its immediate hydrogen or electron acceptor. Still less does it react with oxygen.

The mode of action of dehydrogenases is uncertain; but it is reasonable to suppose that they act like other enzymes and form compounds of greater or less stability with their reactants.[2] Being redox enzymes they have two reactants; the oxidant and reductant; so it may be supposed that they combine with one or other first and that this binary compound then combines with the second reactant to form a triple complex. The attachment of the oxidant to most dehydrogenases is very weak, so that it readily dissociates from the complex and can be totally removed by dialysis. According to the strength of the enzyme-reactant bond, such substances are known as carriers (weak attachment, ready dissociation), or coenzymes (moderate attachment). The known dehydrogenases are specific to one or other of the three oxidants, Coenzyme I, Coenzyme II,[1] or cytochrome-*c*, a carrier. On the reductant side, each dehydrogenase is probably specific for one only, after which it is named. Table 30 is a list of dehydrogenases known to exist in plants.

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[1] Thunberg, 1921, 1928, 1929.

[2] Arguments in favour are developed by Potter and Dubois, 1943; Lu Valle and Goddard, 1948.

Isocitric dehydrogenase is accompanied, perhaps invariably, by the enzyme aconitase, which converts citric acid via *cis*aconitic acid to isocitric. Usually a preparation that will dehydrogenate isocitric acid will also dehydrogenate citric acid, somewhat more slowly.[2] Older records of the occurrence of a citric dehydrogenase[3] are probably to be taken as indicating the presence of aconitase+isocitric dehydrogenase.

Although the number of plant dehydrogenases may be considerable, it is not, apparently, unlimited. In a survey of the amino-acid enzymes we were able to observe dehydrogenation of L-glutamic acid in *Datura*, potato, radish, pea, barley, and onion root tips. *Datura* tips also contained an L-ornithine dehydrogenase, but revealed no evidence of enzymes for alanine, valine, aspartic acid, α-aminovaleric acid, arginine, acetylornithine, lysine, or proline. *Phaseolus* and pea seedlings contain an active glutamic dehydrogenase, but apparently none for glycine, alanine, tyrosine, histidine, aspartic acid, leucine, or proline.[4] Berger and Avery in their detailed investigation of oat coleoptiles found no evidence for the following: sucrose, glucose, lactic, aspartic, tartaric, oxalic, or maleic. Okunuki reports negatives for glucose, fructose, glyceraldehyde, dihydroxyacetone,[5] formic, acetic, and acetaldehyde[6] in lily pollen. Inasmuch as dehydrogenases are possible points of contact between glycolysis and oxidation, such results have importance. The amino-acids enter into the respiration of senescence and starvation (p. 52); but, especially in the later stages, may also be oxidized more

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[1] Coenzyme I is sometimes called diphosphopyridine nucleotide and Coenzyme II triphosphopyridine nucleotide. They differ only in the number of phosphate radicles and otherwise consist of 1 molecule of adenine, 1 of nicotinamide, and 2 of ribose. They are redox bodies by virtue of a fluctuating valency of the N in the nicotinamide ring. As we are concerned with them only in their role of electron transporters, the functional names will be used for simplicity.

[2] Bonner and Wildman, 1946; Whatley, 1951*b*; Berger and Avery, 1943*b*.

[3] Thunberg, 1929; Okunuki, 1939*a*; Wagner-Jauregg and Rauen, 1935; Dann, 1931.

[4] Damodaran and Nair, 1938.

[5] Okunuki, 1940.

[6] Okunuki, 1939*a*.

TABLE 30

Plant dehydrogenases. The enzyme is named after the reduced form in the system it combines with

Reductant	*Hydrogen or electron acceptor*	*Inhibitors*	*Source*	
α-phosphoglycerol	..	..	oat coleoptile	Berger and Avery, 1943
Phosphoglyceraldehyde (supplied as hexosediphosphate)	..	..	cucumber seeds	Thunberg, 1929
	Coenzyme I	..	lily pollen	Okunuki, 1940
	Coenzyme I	M/1,000 iodoacetate	barley	James, Heard, and James, 1944
	..	,,	*Arum* spadix	James and Beevers, 1950
	Coenzyme I	,,	wheat leaves	Waygood, 1950
	Coenzyme I	,,	*Sauromatum* spadix	van Herk, 1937
Lactic acid	..	..	barley leaves	James and Cragg, 1943
	..	..	lily pollen	Okunuki, 1939*a*
	Coenzyme I	..	potatoes	Barron *et al.*, 1950
	..	..	wheat	Waygood, 1950
Ethyl alcohol	..	..	French beans, cucumber seeds	Thunberg, 1921, 1929
	Coenzyme I	..	lily pollen	Okunuki, 1939*a* and *b*
	Coenzyme I	..	oat coleoptile	Berger and Avery, 1943*a* and *b*
	Coenzyme I	..	peas	Adler and Sreenivasaya, 1937
	..	..	spinach leaves	Bonner and Wildman, 1946
	Coenzyme I	..	wheat leaves	Waygood, 1950
	Coenzyme I	..	*Sauromatum* spadix	van Herk, 1937
Succinic acid	..	..	French beans	Thunberg, 1921
	Cytochrome	malonate	lily pollen	Okunuki, 1939*a* and *b*
	..	,,	cucumber seeds	Basu and Karkum, 1943
	..	,,	peas, barley, and cucumber	Das and Gupta, 1941
	Cytochrome	,,	peas	Goddard and Meeuse, 1950
	Cytochrome	,,	seedlings	Damodaran and Venkatesan, 1941
	Cytochrome	,,	seedlings, &c.	Bhagvat and Hill, 1951
	Cytochrome	,,	wheat	Waygood, 1950
	Cytochrome	,,	potatoes	Millerd, 1951

TABLE 30 (*cont.*)

Reductant	*Hydrogen or electron acceptor*	*Inhibitors*	*Source*	
Malic acid .	. .	. .	lily pollen	Okunuki, 1939*a*
	. .	. .	French beans	Thunberg, 1921
	Coenzyme I	oxalo-acetate	oat coleoptiles	Berger and Avery, 1943*a* and *b*, 1944
	Coenzyme I	,,	wheat seedlings	Waygood, 1950
	Coenzyme I	,,	pea seedlings	Goddard and Meeuse, 1950
	. .	,,	spinach leaves	Bonner and Wildman, 1946
Isocitric acid .	Coenzyme II	M/100 pyrophosphate	oat coleoptiles	Berger and Avery, 1944
	. .	,,	spinach leaves	Bonner and Wildman, 1946
	Coenzyme II	,,	chickweed, dead-nettle, elder and charlock leaves	Whatley, 1951*b*
Oxalic acid .	. .	. .	seeds	Thunberg, 1928
Formic acid .	Coenzyme I	M/1,000 HCN	peas	Adler and Sreenivasaya, 1937
	. .	,,	French beans	Thunberg, 1921
	Coenzyme I	,,	French beans, &c.	Davison, 1951
Glutamic acid .	. .	. .	lily pollen	Okunuki, 1939*a* and *b*
	Coenzyme I	. .	oat coleoptile	Berger and Avery, 1943*a* and *b*
	. .	. .	*Phaseolus* and *Pisum* seeds	Damodaran and Nair, 1938
	. .	. .	root tips	James, James, and Beevers, unpublished
	. .	. .	spinach leaf	Bonner and Wildman, 1946
Ornithine .	. .	. .	potato tomato *Datura* } root tips	James, James, and Beevers, unpublished

directly (p. 194). Unfortunately, the plant dehydrogenases have often proved to be fragile enzymes readily destroyed or inhibited during attempted extraction, so that negative results may be difficult to interpret.

The effect of redox dyes on plant respiration

The reduced forms of dyes such as methylene blue are spontaneously reoxidizable in air. On this account it is possible to set up a system reductant-dehydrogenase-coenzyme-methylene blue-O_2 in which there is continuous oxidation of the reductant and continuous consumption of oxygen. Cell-free extracts of *Arum* spadix[1] contain the dehydrogenase-coenzyme complex in exceptional amounts. When first prepared, they also contain considerable amounts of reductant, and additions of methylene blue are immediately bleached. On standing, blue returns at the exposed surface; but is unable to penetrate the body of the liquid. If air is then blown through, oxidation of the reductants goes to completion, and the solution finally becomes blue. Addition of hexosediphosphate renews the bleaching, and oxygen consumption begins again. The above system is then in operation with triosephosphate, derived from the hexosediphosphate, as hydrogen donor. A remarkable feature of these preparations is that, not only is oxygen consumption catalysed, but also the production of CO_2 in equivalent amounts.

If dehydrogenases are active within the living tissues, it should be possible to reproduce this effect, since the dyes are readily taken up and, in catalytic amounts, are not toxic. This has been done with a variety of cells and tissues. The oxygen consumption of *Chlorella pyrenoidosa* is increased by 10^{-3}M methylene blue and other redox dyes. It was claimed that the greatest effect was obtained with the more easily reduced dyes, i.e. those with relatively positive redox potentials, such as methylene blue ($E_0 = +0{\cdot}011$ V) and cresyl blue ($+0{\cdot}047$ V). Neutral red ($-0{\cdot}325$ V), phenyl safranin ($-0{\cdot}252$ V), and indigotetrasulphonate ($-0{\cdot}046$ V) gave little or no effect.[2] A very large effect has been observed with well-washed carrot disks. A concentration of 1/10,000 methylene blue raised the CO_2-output by 360 per cent. The maximum rate was attained after 12 hours and afterwards fell slowly.[3] A similar experiment, usually with 2,6-dichlorophenolindophenol ($E_0 = +0{\cdot}217$ V), is performed as a class routine in the Oxford laboratories. Increases of the same order of magnitude are observed with the CO_2-output of cherry-laurel leaves and disks of belladonna root. It is significant that CO_2-output is increased as well as O_2-intake; it suggests that acceleration of the oxidation, by way of dehydrogenases, increases the normal CO_2-release and hence that dehydrogenase activity really is a normal part of the respiratory mechanism.

PLANT OXIDASES

Plant oxidases may be less numerous than dehydrogenases; but, by reason of greater accessibility, have been much more closely studied. They are usually more robust and readily extracted, and some have been obtained in a fair state of purity. They differ from the dehydrogenases in having firmly attached prosthetic groups of non-protein character, which cannot be removed by dialysis. Except for the autoxidizable flavoproteins, they contain a transition-group metal essential to their functioning, and the oxidases are therefore liable to specific poisoning by traces of metal-binding compounds. The dehydrogenases, except for the irregular formic dehydrogenase of peas, are insensitive to such reagents. Conversely, the oxidases are usually more stable to heat and are not inactivated by thiol reagents such as iodoacetate or by malachite green.

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[1] James and Beevers, 1950.

[2] Genevois, 1929*a* and *b*.

[3] Turner, 1940.

The oxidases are completely specific for their oxidant, which is always molecular oxygen; they will not pass electrons to redox dyes. They are the terminal enzymes of respiration, and can occupy no other position. They are also wholly, or almost wholly, specific on the reductant side. Each oxidase receives electrons from a specific donor, which has the character of a carrier and after which it is named. Three plant oxidases, or possibly groups of oxidases, depending on metals, are sufficiently well defined to be considered in relation to respiratory function; all are widespread in plant tissues; but none is universal. In order of seniority they are polyphenol oxidase, cytochrome oxidase, and ascorbic oxidase.

POLYPHENOL OXIDASE

Palladin's oxidases belonged to this class, the chromogens being the electron donors. The chromogens were not chemically examined in detail; but were realized to have polyphenol characters.[1] The first enzyme of this type to be studied was laccase, discovered by Yoshida[2] in the latex of lac trees, where it is responsible for the initial stage in the darkening and hardening that produces Chinese and Japanese lacquer. Bertrand[3] made the first extensive examination and showed that it would oxidize 'aromatic compounds having at least two —OH or —NH_2 groups'.[4]

Very numerous substances satisfying these requirements occur freely in plants. They include the anthocyanin and flavone pigments, many other glycosides, tannins and tannin derivatives, all of which contain paired hydroxy groups in adjacent positions on benzene rings. The type compound is (pyro)catechol, and the enzyme is alternatively called catechol oxidase. Catechol itself is obtained from a number of plants and several of the simpler derivatives are given in Table 31. All those mentioned after caffeic acid, except urushiol and laccol, may be regarded as simply related to it. Urushiol and laccol are the best defined catechols of *Rhus* latex, but occur in association with a mixture

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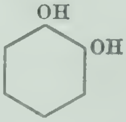
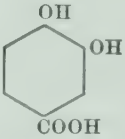
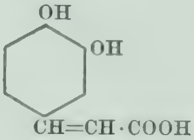
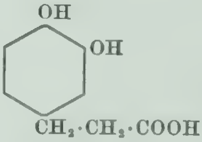
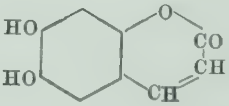
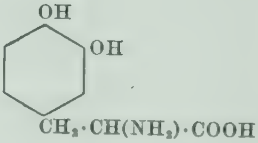
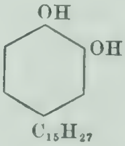
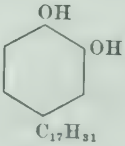
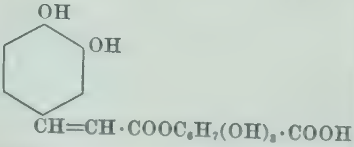
[1] Palladin, 1923.
[2] Yoshida, 1883.
[3] Bertrand, 1894, 1895.
[4] Bertrand, 1896, 1897.

TABLE 31

	Compound	*Isolated from*	
Catechol .		*Populus, Salix, Azara microphylla*	Weevers, 1904 Heatley, unpublished
Protocatechuic acid .		*Vitis vinifera*	Czapek, 1922
Caffeic acid .	 OH OH CH=CH·COOH	*Anthemis nobilis* *Clematis vitalba*	Power and Browning, 1914 Tutin and Clewer, 1914
Dihydroxyphenylpropionic acid .	 OH OH CH₂·CH₂·COOH	*Protea*	Czapek, 1922
Aesculetin .	 HO HO O CO CH CH	Horse chestnut	,, ,,
Dihydroxyphenylalanine, 'dopa' .	 OH OH CH₂·CH(NH₂)·COOH	*Vicia faba*	Guggenheim, 1913
Urushiol .	 OH OH C₁₅H₂₇	*Rhus vernicifera*	Majima, 1922
Laccol . .	 OH OH C₁₇H₃₁	*Rhus orientalis*	,, ,,
Chlorogenic acid . .	 OH OH CH=CH·COOC₆H₇(OH)₃·COOH	widely distributed	Gorter, 1910; Oparin, 1921

of related phenols. Chlorogenic acid is reputed to have a wide distribution in plants and has been related to their polyphenolase action.[1] The specificity of the tests employed is, however, open to some doubt.

Onslow[2] examined about 45 widely distributed plant species for the presence of catechol compounds and polyphenol oxidase. All those that showed the oxidase activity, about 30, contained catechols, the remainder having no polyphenolase also lacked catechols. It is a remarkable thing that more progress has been made with purifying the enzyme than the carrier. Potatoes yield a mixture of polyphenols[3] that have not yet been separated, and *Atropa belladonna*, which also contains an active polyphenolase, gives a mixture which is partially separable on paper chromatograms. It is therefore a difficult matter to determine the specificity of a polyphenolase, and to know whether we have to do with a single enzyme of low specificity or a numerous class with each member adapted to a single polyphenol. Tea enzymes have a higher affinity for tea polyphenols, which have been more thoroughly separated (p. 197), than for catechol or pyrogallol, and oxidize the natural substances fastest.[4] Further, they oxidize their own polyphenols faster than the natural polyphenols of *Eugenia balsamea*; and the *Eugenia* enzyme behaves conversely. These results may indicate polyphenol oxidase specificity, though the enzyme of *Eugenia* has not yet been characterized.[5]

Extracted polyphenolases usually oxidize catechol itself much faster than any of its simple compounds. Initial rates of oxidation by a crude belladonna oxidase were in the ratios catechol 1·0, aesculetin 0·36, adrenalin 0·29, gallic acid 0·06.[6] With a highly purified potato polyphenolase the ratios for dihydroxyphenylalanine, caffeic acid, and protocatechuic acid were all about 0·001.[7] The initial product with catechol is *o*-(benzo)quinone, a powerful oxidizing agent, and it seems possible that the relatively slow oxidation of other polyphenols might be secondary, the very pronounced difference between crude and purified enzymes certainly pointing in this direction.

A very interesting claim is made by Trautner and Roberts for a complex catechol compound that they obtained in small yield from belladonna. They considered that it was not only able to replace catechol as a substrate for belladonna polyphenolase; but that it was 'even superior in its action', a hint of specificity of a particular enzyme for a particular polyphenol. They also considered that, in promoting a continuous oxidation of certain amino-acids under biologically suitable conditions, para-substituted catechols were more stable, more efficient, and smoother working than catechol itself. Unlike the compounds already mentioned, those used by Trautner and Roberts were all of the type

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[1] Gorter, 1910; Oparin, 1921.

[2] Onslow, 1931.

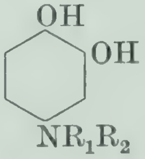
[3] Boswell and Whiting, 1938.

[4] Roberts and Wood, 1950.

[5] Roberts and Sarma, 1940. See p. 196 concerning the nature of the tea enzyme.

[6] From data by James, Roberts, Beevers, and de Kock, 1948; Li and Bonner give similar results for tea polyphenol oxidase.

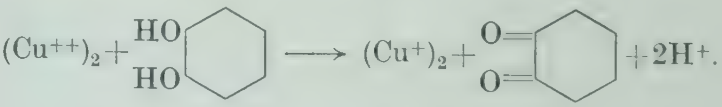
[7] Warburg, 1949.



i.e. having amino groups at position 4.

There is direct evidence that polyphenol oxidases from different sources are not all identical. Three—two of them from higher plants and one from a mushroom—have been obtained in a high state of purity, and show certain specific differences.

Potato polyphenol oxidase was purified in 1937–8 by Kubowitz and studied in some detail, allowing a number of important statements to be made. The enzyme was found to contain copper as a non-dialysable prosthetic group attached to its protein. Its concentration in the purest specimen obtained was 0·165 per cent., and, in a series of purifications, the activity of the preparation was proportional to its copper content. The copper could be removed by treatment with HCN under suitable conditions; the enzyme was then inactive, and activity could be restored by adding about ten times the amount of copper removed. This was the first demonstration that copper is a prosthetic group in biological oxidations. The enzyme was not only inactivated by HCN, but also by the more specific copper reagents salicylaldoxime and diethyldithiocarbamate, and these inhibitions were not reversed by dialysis. It was also confirmed, following earlier work with crude preparations,[1] that the enzyme was inhibited by carbon monoxide and that the inhibition, by contrast with that of iron enzymes, was not reversed by light. A mixture of 90 per cent. CO + 10 per cent. O_2 gave about half the rate of oxygen uptake that occurred if the carbon monoxide was replaced by an inert gas. It was found that an atmosphere of carbon monoxide alone did not combine with the oxidized enzyme; if catechol was then added, CO_2 was absorbed to the extent of 1 molecule for every 2 atoms of copper, and no copper was expelled. It was therefore inferred that CO combined with the reduced enzyme, and that the reacting unit of the enzyme probably contained two atoms of copper. The equation for the oxidation of catechol would then be



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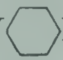
[1] Raper, 1932.

The final product is water; hydrogen peroxide, contrary to earlier belief, is not generated.

Laccase[1] and the mushroom polyphenol oxidase[2] are also proteins with a similar percentage of copper and similar properties. Laccase does, however, show some differences. Unlike the other polyphenol oxidases, it oxidizes *p*-phenylenediamine, H_2NNH_2, faster than catechol; it is accompanied by a blue pigment which is colourless when reduced, and is not inhibited by carbon monoxide at all.

These purified enzymes are all extremely active, the potato one has been calculated to attain an oxygen consumption of 575 cu. mm. O_2/min./mg. enzyme, or $1{\cdot}55 \times 10^7$ cu. mm. O_2/min./mg. copper. It is claimed that potatoes grown on Cu-deficient soils may show virtually no polyphenolase activity; but the enzyme cannot be created merely by adding copper to their extracts.[3]

Monophenols have posed a difficult problem, not yet solved. Some, notably tyrosine, are oxidized by crude polyphenolase preparations, the property disappearing more or less completely during purification. It is still uncertain whether the purification removes a specific monophenolase (tyrosinase) or only traces of *o*-diphenols able to catalyse a secondary oxidation of the monophenols.[4] In either way a redox system could be set up.

Pigment formation

It must have been a very early discovery that many damaged plant tissues slowly develop colours. The brown of a bitten apple, the bright orange of sawn-off branches, the red of the Satan fungus that turns blue on injury, and the blacks of dying beans and pear leaves are familiar examples. These are all consequences of polyphenol oxidase activity, and similar colorations are normal in some healthy tissues; the black spot on bean petals, and the blacks and browns of butterflies' wings, for example.

The *o*-quinones, the primary products of polyphenolase action, are not themselves highly coloured; but in the absence of an efficient reducing system they oxidize further, to give brownish mixtures. *In vitro*, the complete oxidation of a molecule of catechol is found to require 2 atoms of oxygen. Nelson and Dawson observed that during the disappearance of *o*-benzoquinone in water a substance was formed which was treated by the enzyme as catechol. They proposed the following series of reactions,

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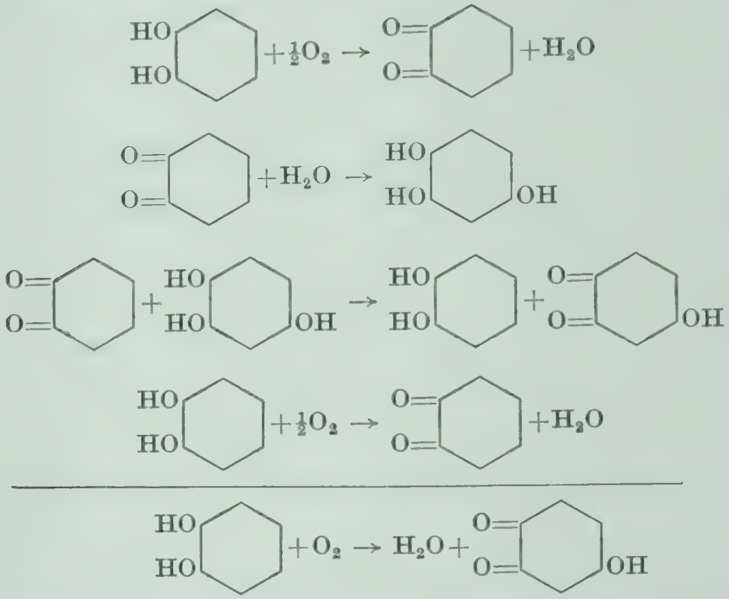
[1] Keilin and Mann, 1939.

[2] Ibid. 1938.

[3] Mulder, 1949, 1950.

[4] For discussions taking opposite sides see Nelson and Dawson, 1944, and Kendal, 1949.



1. fast reaction, catalysed by enzyme.

2. non-enzymatic, fast at pH 7, slow at pH 4.

3. probably instantaneous.

4. as 1.

In sum

the p-hydroxy-o-quinone then condenses rapidly to give the browns observable in an oxidized catechol solution. These are relatively feeble colorations, and much stronger ones arise if amino-acids are present. Amino-acids cause reddy-brown colours; and secondary amines, including the imino-acid proline, give still stronger and more permanent purplish reds. These pigments have eluded a number of efforts to crystallize them, but there is considerable evidence that they are p-amino-o-quinones[1] in which the amino-compound occupies the place taken by the —OH group during the hydration of o-benzoquinone in their absence (Table 32). In tissues containing free amino-acids, discolorations due to injury can arise by such reactions; proline colours are often conspicuous during the wounding of mushrooms and sprouting belladonna stocks. These colours also slowly degenerate to browns as further reaction goes on.

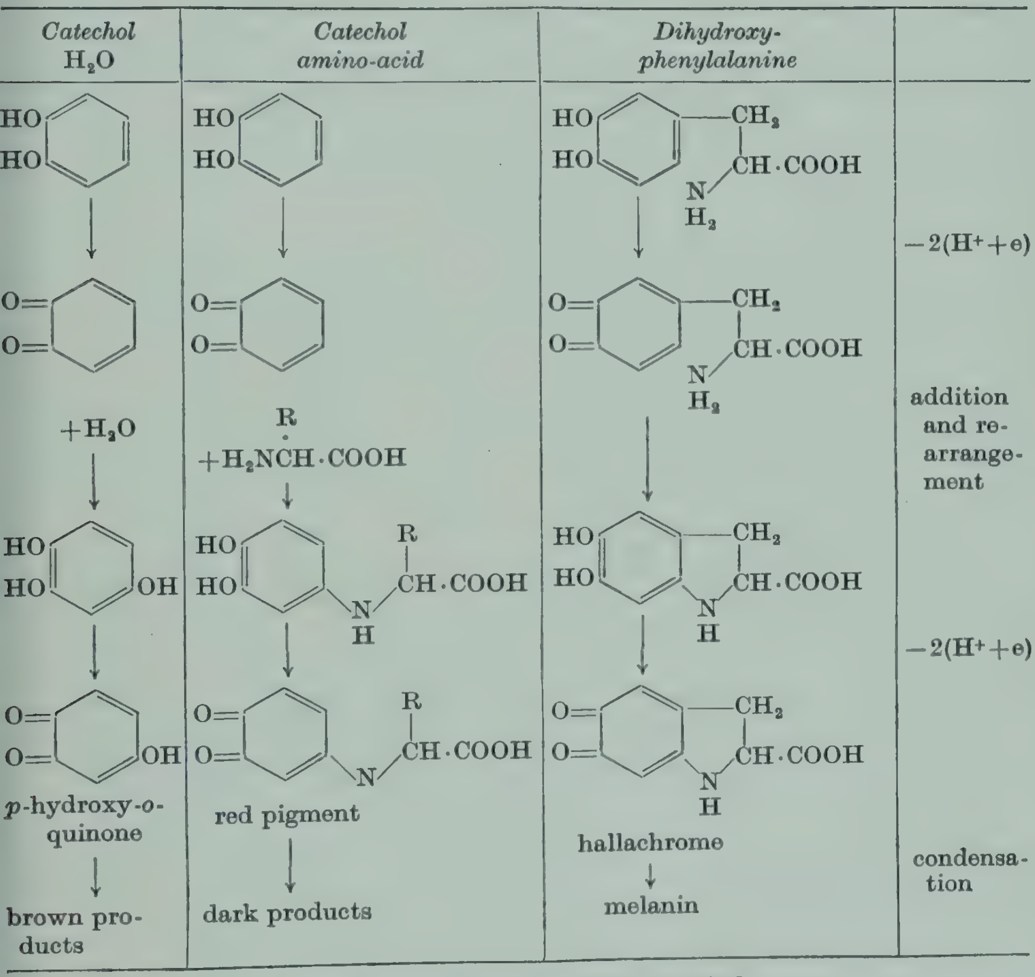
The most important of these colours, because it occurs in normal cells is derived from tyrosine, $HO \cdot \phi \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH$, which combines within its molecule the functions of phenol and amino-acid. The typ of reaction discussed therefore occurs with the formation of indole rin structure. The series of reactions was worked out by Raper[2] using tyrosinase preparation from meal worms, and has been a guide to al later investigation of these reactions. Tyrosine is first oxidized to th diphenol, dihydroxyphenylalanine. The subsequent steps are show in Table 32 collaterally with those of catechol and catechol+amino-acid. Hallachrome is a red pigment structurally analogous to the amino-benzoquinone pigment. It is stable in the worm *Halla* and perhaps in butterflies' wings;[1] but it also undergoes further changes to give the black condensation products called melanin, responsible, for example, for blackening in potatoes.[2] The diphenolic amine, adrenaline, $(HO)_2 \cdot \phi$, $CHOH \cdot CH_2 \cdot NH \cdot CH_3$, is also known to give a similar series of reactions with polyphenolases, with production of red adrenochrome.[3]

---

[1] James, Roberts, Beevers, and de Kock, 1948; Beevers and James, 1948; Jackso and Kendal, 1949; Trautner and Roberts, 1950.

[2] Raper, 1926, 1927, 1928.

TABLE 32


Catechol
H₂O
Catechol
amino-acid
Dihydroxy-
phenylalanine
+H₂O
+H₂NCH·COOH
R
p-hydroxy-o-quinone
brown products
red pigment
dark products
hallachrome
melanin
−2(H⁺+e)
addition and re-arrangement
−2(H⁺+e)
condensation

After Trautner and Roberts, modified.

Oxidation of amino-acids

Amino-acids are oxidized by extracted polyphenol oxidases in the complete absence of dehydrogenases, though at widely differing rates. Particularly susceptible are glycine and dipeptides in which its amino-group remains free. For each molecule of amino-acid oxidized, approximately 1 molecule of ammonia is released, and 1 molecule of the corresponding α-keto-acid formed.

---

[1] Ford, 1945. [2] Mulder, 1949. [3] Green and Richter, 1937.

$$R\cdot CH\cdot NH_2\cdot COOH+\tfrac{1}{2}O_2 \rightarrow R\cdot CO\cdot COOH+NH_3.$$

Glyoxalic acid,[1] pyruvic acid, and α-keto-δ-amino-valerianic acid[2] have been identified as the oxidation products of glycine, alanine, and ornithine respectively. Other amino-acids were oxidized too slowly for their products to be obtained.

The mechanism of the reaction *in vitro* is rather complex, because pigment formation takes precedence of oxidation. Probably the oxidizing agent is not *o*-quinone; but the quinonoid red pigment which is also capable of accepting hydrogen or an amino-compound. A cycle of reactions starting with such a combination, and leading to continuous release of ammonia and keto-acid, has been proposed by Trautner and Roberts. Inside the cell the situation may be simpler. Some amino-acid dehydrogenases exist, which might transfer hydrions and electrons by way of Coenzyme I to the quinone without requiring an amino-acid-quinone combination. This seems particularly likely with glutamic acid, for which an active dehydrogenase is known, and which is only sluggishly oxidized by belladonna polyphenolase. It also has to be remembered that amino-acids are oxidized in tissues that possess no polyphenolase, so that in them some other mechanism must exist.

Tea 'fermentation'

Autolysis is set up in the plucked leaf by wilting it until it is no longer brittle, and repeatedly passing it between rollers. The pressure does not break up the cell walls; but causes enough shearing to disorganize the protoplasm. This allows polyphenols accumulated in the vacuole to penetrate to the oxidases, and probably also inactivates dehydrogenases by dilution of their coenzymes. The leaf first becomes red-brown, and finally darkens until, after 3–4 hours, further action is stopped by drying in a blast of hot, dry air which reduces the moisture content to about 4 per cent. In green tea these changes are prevented by an earlier inactivation.

It has long been recognized that the colour changes indicate oxidation of the tea polyphenols and, in spite of its name, the process is independent of intrusive micro-organisms; it cannot be prevented by bactericides, and there is no relation between the rate of darkening and bacterial counts. The reactions can, on the other hand, be reproduced by enzymes extracted from the leaf itself.[3]

The tea oxidase is active with catechol, and the *o*-quinone formed has been isolated in the presence of aniline as crystalline dianilino-*o*-benzoquinone.[1] Applying the same technique to tea catechins also produced insoluble anilinoquinones, although they could not be crystallized in appreciable quantities. Oxidation appears not to proceed beyond formation of the *o*-quinone; but this causes rapid condensation to start so that the simple tannins become more complex.[2] The oxidizing enzyme that sets this chain of reactions in progress is a polyphenolase, part of which is readily extracted and part not.[3] The difficulty of extracting the second fraction may be due to its location in chloroplasts rather than to any intrinsic differences in the enzyme.[4] The readily extractable portion has been considerably purified, and among the more active preparations there was proportionality between activity and copper content; the best having 0·08 per cent. copper. Like other polyphenolases, it was more resistant than peroxidase and cytochrome oxidase to cyanide inactivation and showed high specificity for *o*-hydroxyphenols. The most rapidly oxidized substances are pyrogallols, and monophenols are not oxidized.[5]

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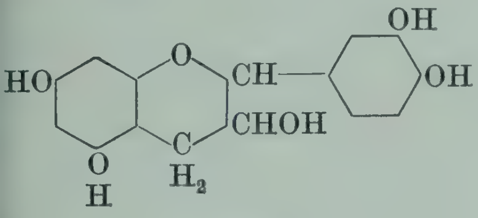
[1] James *et al.*, 1948.

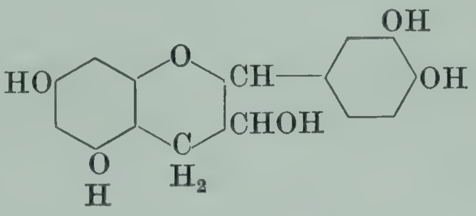
[2] Beevers and James, 1948.

[3] Roberts, 1942.

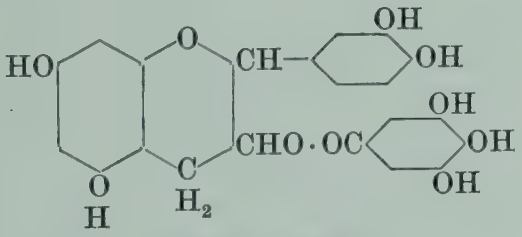
The natural polyphenols of tea are probably flavanols and their galloyl esters.



epicatechin



gallocatechin



epicatechin gallate

By the application of partition[6] and paper chromatographies,[7] separation of the polyphenols has been carried to considerable lengths, and the greater part of the mixture accounted for. Among those found in fresh leaf are catechin, epicatechin, gallocatechin, epigallocatechin, gallic acid, and galloyl esters of the catechins.[7] These are oxidized much more rapidly than catechol and have much higher affinities for the enzyme. The K_m for tea catechin is estimated to lie at 0·001–0·002 M and that for a purified Merck catechin at the same level, whereas the K_m for catechol is about 0·025 M.

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[1] Lamb and Sreerangachar, 1940.

[2] Roberts, 1942.

[3] Sreerangachar, 1943.

[4] Roberts, 1941; Li and Bonner, 1947.

[5] Lamb and Sreerangachar, 1940; Li and Bonner, 1947; Roberts and Wood, 1950.

[6] Bradfield, 1946; Bradfield, Penny, and Wright, 1947; Bradfield and Penny, 1948.

[7] Roberts and Wood, 1951.

The dependence of the 'fermentation' upon a Cu-enzyme is confirmed by its susceptibility to poisoning by dieca (diethyldithiocarbamate) and BAL (British anti-Lewisite).[1] It is analogous in its inception to the pigment formations of the previous section. It results from a traumatic enhancement of polyphenolase activity leading to formation and condensation of *o*-quinones. The abnormal activity depends on freer access of polyphenols to the enzyme. Reduction mechanisms in the tissues are unable to keep pace with the rapid build-up of *o*-quinones and so condensations supervene. The same *o*-quinones, produced at lower rates in the normal cells, might conceivably be hydrogen acceptors in the respiratory sequence; they can, for example, oxidize ascorbic acid. At present there is little to indicate whether they do in fact play any such role.

o-Diphenols as carriers

In all the preceding, diphenols catalyse reactions that are running down rapidly to completion. The activity is induced artificially by mechanical damage or treatment with chloroform; or, naturally, by advancing senescence. It is, in short, an autolysis. It causes enhanced oxygen uptake without equivalent CO_2-output, and is not an acceleration of normal respiration.

Having in mind the evidence for the ready entry of oxygen into potato tissues (p. 144), it is difficult to suppose that potato polyphenol oxidase is normally oxygen starved; and the senescent browning of apple tissues is associated with an increased skin resistance to oxygen entry. We can only suppose that the autolytic reactions start with greater access of polyphenols to the enzymes. We have no evidence that the polyphenols rapidly consumed during browning are the normal substrates, which may be present only in catalytic amounts. Hydrolysis is a factor. Aesculetin (p. 191) is rapidly oxidized by polyphenolase; but its glycoside, aesculin, is not attacked. It is possible that substrates, present in traces, might be 'buffered' in such a way.

The most significant observation is that the complete system, diphenol+enzyme, but not the enzyme alone, will continuously oxidize such important carriers as Coenzyme I, Coenzyme II, and ascorbic acid. This results from the activity of the *o*-quinones as hydrogen acceptors and does not require a second enzyme.[2] The following system has been assembled *in vitro* with potato,[1] apple,[2] belladonna,[3] tea,[4] and mushroom[5] polyphenoloxidases.

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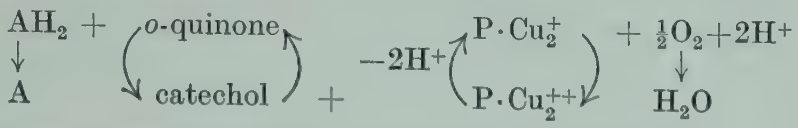
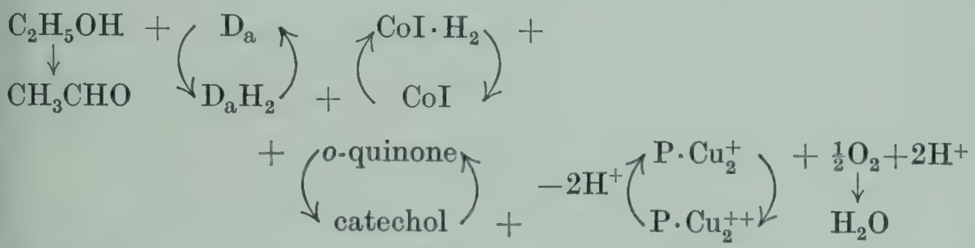
[1] Roberts and Wood, in the press.

[2] Kubowitz, 1937*b*; Dixon and Zerfas, 1940; Warburg, 1949.



AH_2 represents ascorbic acid, $P\cdot Cu_2^+$ and $P\cdot Cu_2^{++}$ reduced and oxidized enzyme respectively. A similar system, substituting tea tannins for catechol was also active.[4]

Longer chains have been established with the coenzymes and appropriate dehydrogenases. Alcohol has been oxidized with potato polyphenolase[6] as follows.



D_a and CoI stand for alcohol dehydrogenase and Coenzyme I respectively. Coenzyme II has been similarly employed with a polyphenoloxidase system to oxidize hexosemonophosphate. The dehydrogenase has been obtained from yeast, though not, apparently, from higher plants, and requires Coenzyme II. The activity of the polyphenoloxidase can even be examined more accurately with the complete system than with catechol only, since no *o*-quinones accumulate to introduce complexities.[7]

It thus seems in theory possible for polyphenol oxidases to act as terminal oxidases in the transport of electrons from glycolysis products to oxygen. Whether they actually do, and if so to what extent, cannot be properly discussed before alternative systems have been described.

THE CYTOCHROME SYSTEM

In 1924 Warburg advanced the theory that 'the reaction of divalent iron is really that reaction by which molecular oxygen most often reacts in biochemistry'.[8] It was based on experiments with model systems containing iron in complex combination on charcoal. These were found to oxidize amino-acids vigorously; but to be inhibited by narcotics, which displaced the amino-acids from the surface of the charcoal. Most striking of all, the oxidation was inhibited by cyanide when the ratio of adsorbed cyanide to adsorbed amino-acid was as low as 1/70. From this came the conception of the specific inactivation of active centres (prosthetic groups), which has since been so profitably applied to the oxidases. Further support came for the iron theory, when it was found that the apparently spontaneous oxidation of the thiol amino-acid cysteine was really catalysed by trace impurities of iron salts.[1] The theory envisaged a respiration enzyme into which an iron complex was bound; but admitted the possibility of similar action by other metals with specific mention of copper. This was regarded as exceptional and as 'lying outside the scope of a general Theory of Respiration'. Emphasis was laid on the fact that iron was the autoxidizable part of the enzyme and that nothing else in the cell reacted with oxygen itself. With the controversy that developed round this idea we need now have nothing to do.

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[1] Kubowitz, 1938.
[2] Johnson and Zilva, 1937.
[3] James *et al.*, 1948.
[4] Sreerangachar, 1943.
[5] Keilin and Mann, 1938.
[6] Kubowitz, 1937*b*.
[7] Kubowitz, 1937*a*.
[8] Warburg, 1924, 1925.

MacMunn published in 1885 the results of a spectroscopic examination of numerous animal tissues. He wrote that they all, 'from echinoderms to man', possess haems, which are iron-containing compounds. He considered the haems to be conjugated with proteins, and showed that they were capable of alternate oxidation and reduction. This was possible because in the reduced form they showed a well-defined absorption spectrum of four bands in visible wave-lengths which, upon oxidation, disappeared. These changes could be followed particularly well in the wings of flying insects. The position of the bands did not vary a lot in different tissues and averaged round 605 mμ (yellow-red), 567 mμ (green), 550 mμ (green), and 522 mμ (blue-green). They were described as 'strong, strong, very strong and weak' respectively. He called the pigments histohaematins. He was able to extract a part of them, and found that outside cells they were no longer capable of oxidation by atmospheric oxygen. On account of their properties in the cell he thought they must be respiratory.

The time was not ripe for this idea, and it was argued down, until in 1925 Keilin rediscovered the histohaematins and rechristened them cytochromes. This name was less descriptive, but less of a mouthful and is now always used. Keilin showed that the four absorption bands were attributable to three compounds, which he named cytochromes *a*, *b*, and *c*. The fourth absorption band is probably common to all three cytochromes, with cytochrome *c* making much the strongest contribution. It is sometimes resolvable into three closely-placed bands. Deeper in the blue, all three cytochromes show another absorption zone,[2] so that each has three bands, α, β, and γ. Further examination of the band at 605 mμ showed that it was not homogeneous and that it was liable to shifts to about 630 mμ or 590 mμ. The shifts were attributed to lability of cytochrome a allowing it to be easily modified to a_1 (590 mμ) or a_2 (630 mμ).[1] Although centring at 605 mμ, the a band is wide and has a component at 600 mμ which on reaction with CO moves to 592 mμ, other cytochrome bands not being affected. The 592 mμ band was associated with a second at 448 mμ, in the deep blue, and the substance responsible was called cytochrome a_3.[2] Recently two further cytochromes, lettered b_3 and f, have been recognized in plant tissues.[3] The positions of the various bands and some other important properties of the cytochromes are listed in the following table.

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[1] For English summary see Warburg, 1949.

[2] Warburg and Negelein, 1931*a* and *b*,

TABLE 33

	Cytochrome					
	a	a_3	b	b_3	f	c
absorption bands α	605	600	564	560	555	550
,, ,, β	?	?	530	529	533	521
,, ,, γ	452	448	433	. .	. .	417
reaction with CO .	none	combines	none	none	none	none
,, ,, HCN	none	combines	none	. .	. .	none
,, ,, O_2 .	none	autoxidizable	slightly autoxidizable	autoxidizable	none	none pH 4–12
with succinic system	reduced	not easily reduced	. .	. .	. .	reduced
redox potential, E_0	+0·29 V	. .	−0·04 V	. .	+0·34 V	+0·27 V
solubility . .	insoluble	. .	insoluble	soluble	insoluble	soluble

Distribution of the cytochromes

Many organisms have now been examined spectroscopically for cytochromes, and it is clear that their distribution is very wide indeed. Among bacteria, one or more are usually present, exceptions being some streptococci, that are facultative anaerobes, and the *Clostridia*, that are strict anaerobes.[4] Among animals, a, b, and c all appear to be wellnigh universal; and yeast is a relatively rich source. Cytochromes are also widely distributed in the higher plants, but spectroscopic examination is made difficult by a very low concentration and often by the presence of other pigments. Keilin's original paper reported occurrence in a considerable range of colourless plant parts, which was confirmed and widened by Hill and Bhagvat.[5] The positions of the bands observed with some plant tissues are shown in Fig. 63. It is noteworthy that the majority of the records are for embryonic or seedling materials. While it was possible to observe cytochrome bands in barley embryos, none could be found in older, although quite active, root tips.[1] An exhaustive examination of the adult tea plant gave no indications of cytochrome in any part.[2] These negative observations might well be set aside on the grounds of low concentration if they stood alone; but they will need to be viewed again in the light of other information (p. 222). Cytochrome *f* is exceptional; unlike the other cytochromes, it is found in mature leaves and especially in their chloroplasts. It is named *f* (for Latin *frons*) on this account.

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[1] Keilin, 1934.

[2] Keilin and Hartree, 1939, using ox heart.

[3] Hill and Scarisbrick, 1951.

[4] Stephenson, 1949.

[5] Bhagvat, 1939; Hill and Bhagvat, 1939; Bhagvat and Hill, 1951. See also Lundegårdh, 1951*a* and *b* for young roots of wheat and maize.

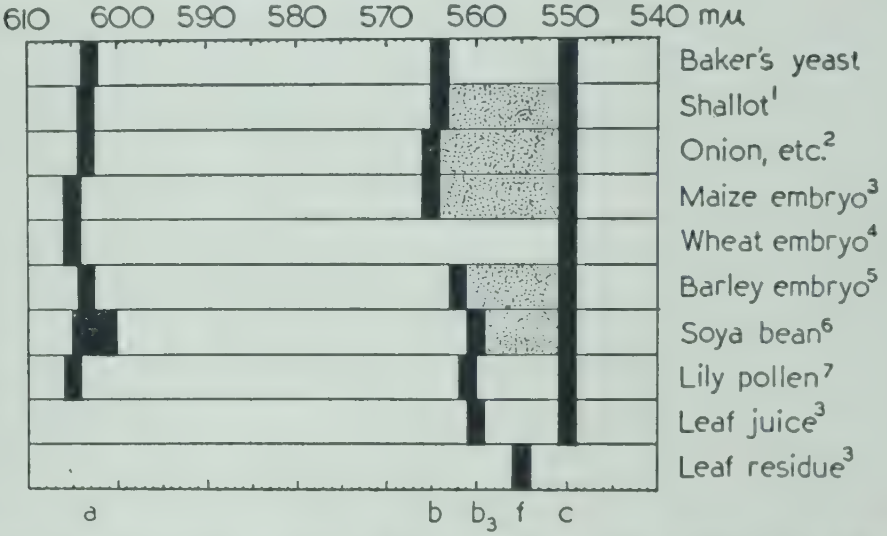

FIG. 63. α-absorption bands of cytochromes in plant tissues. The shading between *b* and *c* bands is due to haemochromogens. 1. Keilin, 1925; 2. Bhagvat and Hill, 1951; 3. Hill and Scarisbrick, 1951, juice from broad bean and other leaves. Residue after acetone extraction of elder leaves. 4. Brown and Goddard, 1941; 5. James, unpublished. Mikhlin and Kolesnikov give wide bands covering the same areas. 6. Yakashuji, 1934, also green leaves and algae. 7. Okunuki, 1939*a*.

Cytochrome *c*

This component is more stable than the others and has been isolated from yeast[3] and purified, initially, from ox heart.[4] It is a conjugated protein and the purest preparations are stated to contain 0·43 per cent. iron and to have a molecular weight of 13,000, including 1 haem molecule. The haem is very tightly bound to the protein, probably by a thiol ether link through cysteine radicles in the protein. The link cannot be broken and restored, like the corresponding link in haemoglobin.[5]

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[1] James, 1946; Mikhlin and Kolesnikov, 1947.

[2] Sreerangachar, 1943.

[3] Keilin, 1930.

[4] Theorell, 1936; Keilin and Hartree, 1937, 1938.

[5] Theorell, 1938, 1943.

Extracted cytochrome *c* does not react with molecular oxygen; it can readily be reduced and its absorption bands correspond exactly with the c_α and c_β bands observed in the living tissue.

Cytochrome *c* has also been got out of plants. Yakashuji (1934) made an extraction from the seaweed *Porphyra tenera* obtaining 'a very concentrated clear red solution of cytochrome *c*' with strong α and β bands at 550 and 520 mμ. Addition of 3 volumes of acetone threw down the cytochrome with other proteins. The yield was 1 gm. from 5 kilos of seaweed. A much purer preparation has been obtained from wheat embryos by Goddard (1944). The methods used with heart extractions could not be applied successfully; and extraction with M/10 K_2HPO_4 was substituted. The product was identical with ox heart cytochrome. Absorption bands α and γ were at 550 and 417 mμ, and behaviour towards oxidizing and reducing systems was the same. The yield was 4·6 mg. per kilo wheat germ. Hill and Scarisbrick have isolated cytochrome *c* from broad bean leaves by an ammonium sulphate method; they estimated the concentration in the fresh leaf to be of the order of 10^{-6} M.

Cytochrome oxidase

The cytochromes, apart from a_3 and b_3, do not react readily with oxygen under biological conditions. The dilemma of MacMunn on finding that extracted histohaematins were no longer spontaneously oxidizable therefore remained. After some discussion, it was settled that the enzyme oxidizing cytochromes *in vivo*, i.e. cytochrome oxidase, was identical with the iron-containing respiratory enzyme postulated by Warburg.[1] Unfortunately, it has resisted all efforts to extract it into pure solution, and is still only known in suspensions. It is, however, extremely sensitive to inhibition by HCN, H_2S, and sodium azide. It is also inhibited by CO in the dark, the inhibition being reversed by light. This indicates that the metal concerned must be iron, since only iron-carbonmonoxide complexes are photolabile. It also provides a means for determining the absorption spectrum of the cytochrome oxidase-CO compound. To be effective the light must be absorbed, and the degree of effectiveness is found to vary with wave length. Warburg,[2] by measuring the amount of light-reversal in different wave-lengths, was able to plot the absorption spectrum of the compound, a spectrum which no one had ever seen. He found it to be of similar type to those of the cytochromes and other haem compounds, with α, β, and γ bands at 590, 540, and 430 mμ respectively, and other bands deeper in the violet. It was found later that the absorption spectrum of the cytochrome a_3-CO compound also had bands at 590 and 430 mμ, the β

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[1] Warburg, 1927; Keilin, 1927; see also Warburg, 1949.

[2] Summarized account in Warburg, 1949.

band being too faint to be seen. Since a_3 was, further, the only cytochrome to combine with carbon monoxide, HCN, and oxygen, the identity of cytochrome a_3 and the enzyme seemed established. Nevertheless, difficulties accumulated; cytochrome a_3 remained inhibited by CO in the light, it was not reduced by reduced cytochrome c, it was absent from some bacteria whose respiration was inhibited by CO and HCN, and, finally, the other cytochromes could be oxidized in cells in spite of the CO band of cytochrome a_3 being visible. It is still, therefore, uncertain whether the absorption spectrum of cytochrome oxidase has actually been seen.

Extracted cytochrome oxidase is usually identified by its ability to catalyse the oxidation of suitable substrates, such as hydroquinone and *p*-phenylenediamine, by way of cytochrome *c*. The cytochrome is added only in catalytic amounts and, in the presence of the enzyme, continuous oxygen consumption at the expense of the artificial substrate is set up. On this basis, cytochrome oxidase is obtainable from a long list of plants. The survey undertaken by Bhagvat and Hill is summarized in Table 34; confirmations and additions are obtainable from other sources.[1] Again, as with the cytochromes themselves, young

TABLE 34

Data of Bhagvat and Hill

Plant material	*Cytochrome a, b, c*	*Cytochrome oxidase*	*Succinic dehydrogenase*	*Polyphenol oxidase*
Barley, without endosperm	+	+	+	—
Wheat, without endosperm	+	+	+	—
Maize, without endosperm	+	+	+	—
Peas, cotyledons . .	+	+	+	—
Runner bean, shoot . .	+	+	+	—
,, ,, cotyledons .	+	+	+	—
Broad bean, shoot . .	+	+	+	—
,, ,, cotyledons .	+	+	+	—
Butter bean, cotyledons .	+	+	+	—
Dolichos, cotyledons .	+	. .	. .	+
Golden butter bean, shoot	+	+	+	—
,, ,, ,, cotyledons	+	+	+	—
Leek, bulb . . .	+	+	+	—
Onion, bulb . . .	+	+	+	—
Dandelion, root . .	+	+	+	+
Sea-kale, root . . .	+	+	+	—
Parsnip, root . . .	+	. .	. .	+
Sugar beet, root . .	+	. .	. .	+
Rhubarb, flower buds .	+	+	+	—

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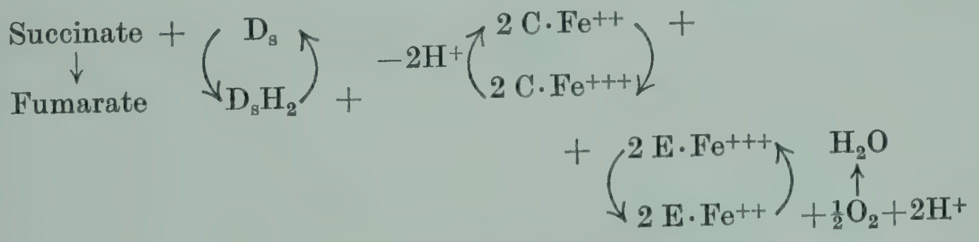
[1] Wheat embryos (Goddard, 1944); wheat seedlings (Waygood, 1950); barley embryos (James, 1946; Mikhlin and Kolesnikov, 1947); oat seedlings (Albaum and Eichel, 1943; Albaum, Donelly, and Korkes, 1942; Bonner, 1948); oranges (Hussein, 1944); chloroplasts of spinach (Rosenberg and Ducet, 1949); pea seedlings (Goddard and Meeuse, 1950); potatoes (Levy and Schade, 1948; Goddard and Holden, 1950).

and even embryonic materials are conspicuous. There are particularly interesting records for oat[1] and wheat[2] seedlings in which cytochrome oxidase activity was demonstrable for about the first 70 hours of germination, but thereafter undetectable. Barley embryos yield active preparations of cytochrome oxidase; but not 12-day-old roots.[3] In a very thorough examination of tea oxidases, Sreerangachar[4] could not obtain any cytochrome oxidase, and the very active system of *Arum* spadix is also unable to co-operate with cytochrome *c*.[5] Bhagvat found no cytochrome oxidase in parsnips. Negative results are open to the suspicion that they are due to an inhibitor gaining access to the enzyme during extraction. Hill and Bhagvat found it desirable to remove most of the suspended matter by centrifuging at 2500 r.p.m. before testing the enzyme. This and other precautions were observed in the examples cited.

Cytochromes as carriers

If the cytochromes are serially arranged in an electron-transporting chain, they might be expected to operate in the order predicted by their redox potentials (Fig. 62, p. 183), i.e. the electrons would pass from $b \rightarrow c \rightarrow a \rightarrow$ oxidase $\rightarrow O_2$.[6] Whether they do so travel must be regarded as still uncertain, and in what follows we may be content to write cytochrome as meaning one or more constituents acting singly or in some corporate manner.

Cytochrome and its oxidase can be used to establish oxidation chains *in vitro*, and the cytochrome used is then of necessity cytochrome *c*. The most familiar such system is the 'succinic oxidase' chain. Succinic dehydrogenase appears to need no coenzyme as mediator between it and the cytochrome; but may have a built-in prosthetic group. It has been obtained in association with cytochrome oxidase from a number of plant tissues (Table 30, p. 186) and the following system set up



where D_s = succinic dehydrogenase and C = cytochrome *c* and E = cytochrome oxidase respectively. Hydrions are lost in the first or second reaction and acquired in the last. A point to be noticed is that the oxidation of one succinic acid molecule requires 2 cytochrome molecules and probably 2 oxidase molecules (as distinct from polymers),[1] and reduces half a molecule of oxygen.

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[1] Albaum and Eichel, 1943.
[2] Waygood, 1950.
[3] James, unpublished.
[4] 1943.
[5] James and Beevers, 1950.
[6] Ball, 1938.

For the reduction of a complete molecule of oxygen the co-operation of 4 cytochrome molecules transferring 4 electrons is needed. There is no evidence of the intermediary formation of hydrogen peroxide. Oxidation of succinate is usually slow *in vitro* compared with the oxidation of *p*-phenylenediamine and other artificial substrates; but it is likely that succinate does not react with cytochrome *c* in the intact cell but with cytochrome *b*.

Longer reaction chains involving cytochrome have also been put together. It has been shown that cytochrome may co-operate with Coenzymes I and II and so connect with a wide range of dehydrogenases. The reaction between cytochrome and the coenzymes is catalysed by flavoprotein enzymes, now usually called cytochrome reductases. Using a reductase with cytochrome *c* and cytochrome oxidase extract from wheat germ, Goddard assembled a system oxidizing glucose-6-phosphate by way of its dehydrogenase, Coenzyme II, cytochrome reductase, cytochrome *c*, and cytochrome oxidase to oxygen. The glucose-6-phosphate dehydrogenase was not obtained from plants, and is not known to occur in them.

THE ASCORBIC SYSTEM

On account of its antiscorbutic role in human nutrition, ascorbic acid has been much sought after. Earlier identifications were biological, i.e. by means of a guinea-pig scurvy test; more recently, immediate reduction of 2,6-dichlorophenolindophenol under stated conditions has been taken to indicate its presence, and comparisons between the two methods give consistent results. Identification has rarely meant the isolation of the crystalline substance.

Many fruits have particularly high contents of ascorbic acid, e.g. walnuts[2] up to about 2 per cent., rose hips 0·5 per cent., and black currants[3] 0·3 per cent. of the fresh weight. Resting seeds have little or none, but appreciable quantities appear immediately upon germination,[4] and there is also an increase in sprouting potatoes.[5] Lack of light much reduces the amount of ascorbic acid formed.[6] The molecular structure suggests a fairly direct formation from a hexose, and, of a large number of substances given to isolated pea embryos, only hexoses led to formation of ascorbic acid.[1] The isolation from *Phaseolus mungo* of an enzyme converting aldohexoses to ascorbic acid has also been claimed.[2]

---

[1] Warburg, 1949, pp. 145–6.

[2] Harris and Olliver, 1942.

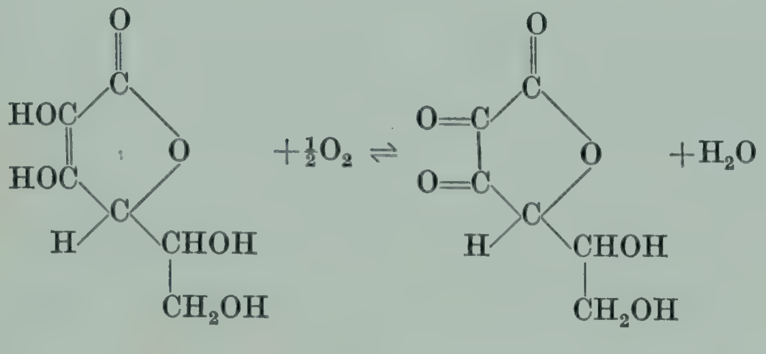
[3] Wokes and Melville, 1948.

[4] Matsuoka, 1943, rice; Bonner and Bonner, 1935; Harris and Ray, 1935, peas and oats.

[5] Pett, 1936; Kroner and Steinhoff, 1937.

[6] Matsuoka, 1943, rice; Harding, Winston and Fisher, 1943, oranges; Sugawara, 1939, various seedlings; Reid, 1938, cowpeas.

Ascorbic acid is reversibly oxidized to dehydroascorbic acid



but dehydroascorbic acid is very unstable above pH 5, so that neutral or alkaline aqueous solutions turn brown in a matter of minutes, with the ultimate formation of oxalic and threonic acids. Ascorbic acid itself is not autoxidizable in pure solutions which are strongly acid, or within the normal acidity range of plant saps lying below pH 7.

All investigators are agreed that in normal cells nearly all the ascorbic acid is present as such, that is, in the reduced form. It is possible that the instability of dehydroascorbic acid debars it from accumulating, and there are very rapid losses in chopped or damaged tissues in which the equilibrium swings to the right. It has been shown that the loss of ascorbic acid is likely to be greater from chopped greenstuffs than from boiled ones owing to heat-inactivation of the enzymes in boiling.[3] It is important that measurable amounts of dehydroascorbic acid are nevertheless present in many tissues, and that the proportions of reduced and oxidized forms vary with occasion. This emphasizes the reversibility of the system within tissues, and is confirmed by observations of reduction of dehydroascorbic acid injected into leaves.[4] Since damage leads to rapid oxidation, it is obviously difficult to decide whether any dehydroascorbic acid is normally present in the intact tissue, and by careful technique Harris and Olliver reduced the possible amount to about 1 per cent. of the total ascorbic acid in half a dozen tissues examined. Potatoes yielded higher ratios up to 6 per cent., and later work[5] has shown ratios up to 10–15 per cent., varying with conditions in ways at present inexplicable. The ratio also varied considerably during starvation of detached Sudan grass and Kikuyu leaves,[1] due to more rapid removal of ascorbic acid. Under nitrogen, rapid loss of ascorbic acid was accompanied by actual increase of dehydroascorbic, which afterwards slowly disappeared also. This is interesting as showing that oxygen is not the only ultimate H-acceptor for ascorbic acid. Apples appear to be the extreme examples with about 55 per cent. of the total as dehydroascorbic acid in the earliest stages, diminishing to 5–6 per cent. towards the end.[2] It is not known how these large amounts of dehydroascorbic acid, equivalent to about 8 mg. per 100 gm. of fruit, are stabilized in the tissues.

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[1] Ray, 1934.

[2] Rudra, 1943.

[3] Harris and Olliver, 1942.

[4] Citations by Crook and Morgan, 1944.

[5] Barker and Mapson, 1952.

Oxidation of ascorbic acid

Although ascorbic acid is not autoxidizable, its oxidation is very readily catalysed in air. Besides hydroxyl ions, many di- and trivalent metals are effective, particularly copper. The traces in water distilled from copper stills are sufficient to cause perceptible oxygen consumption and, in all experiments with the ascorbic system, use of glass-distilled water is essential. The copper in crude plant extracts containing proteins is partly removable by dialysis against water; but part, such as the prosthetic copper of oxidases, is not. The loosely attached dialysable copper is capable of catalysing ascorbic acid oxidation; but, in general, is less active than the equivalent amount of free cupric ion. Artificial copper proteinates show some of the characteristics of an oxidase, having a pH optimum, being thermolabile, and showing similar sensitivity towards inhibitors.[3] Unlike the oxidases they lose all their copper on dialysis and have a lower activity than the equivalent free copper. Inorganic systems oxidizing ascorbic acid may be thermolabile;[4] but they differ from the enzymatic in being inhibited by amino-acids, proteins, and sodium chloride;[5] and particularly in their lack of specificity. Purified ascorbic oxidases do not oxidize phenols, *p*-phenylenediamine, and other compounds liable to oxidation by cupric ions,[6] and are limited to *l*-isomers.[7] The enzymatic oxidation stops when one atom of oxygen has been consumed per molecule of ascorbic acid, whereas the free ionic oxidation proceeds beyond this point.[8]

It is possible that some oxidation of ascorbic acid is catalysed in tissues by Cu^{++} ions either free or loosely attached to non-specific proteins. The general nature of metabolic organization does not suggest that such a haphazard arrangement is likely to be a regular feature. At least four enzyme systems are known to be capable of participating;

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[1] Wood, Cruikshank, and Kuchel, 1943.
[2] Zilva, Kidd, and West, 1938.
[3] King, 1939; Meiklejohn and Stewart, 1941.
[4] Lampitt and Clayson, 1945.
[5] Stotz, Harrer, and King, 1937; Stotz, 1940; Mystokowski, 1942.
[6] Dodds, 1948.
[7] Johnson and Zilva, 1937.
[8] Dawson, 1950.

peroxidase in the presence of hydrogen peroxide;[1] polyphenolase in the presence of polyphenols (p. 198); the cytochrome system,[2] and a direct ascorbic oxidase.

Ascorbic oxidase

In the presence of the other systems, the unambiguous demonstration of the direct oxidase is not very easy. It is consequently best known where at least the polyphenolase is absent, as in parts of the brassicas, cucurbits, and cereals. Preparations from young barley leaves, for example, show little or no polyphenolase activity, and addition of catechol does not accelerate the oxidation of ascorbic acid.[3] The enzyme is particularly abundant in some cucurbits and has been considerably purified. Purified preparations always contain copper. Meiklejohn and Stewart found that, after prolonged water dialysis, the cucumber enzyme oxidized ascorbic acid proportionally to the amount of copper it contained. After further dialysis against M/100 HCN, activity was totally lost, and could be restored by adding copper sulphate, and dialysing against water to remove any excess. By adding a tenfold excess of copper, activity was restored almost to its original level. The addition of an initial excess is probably needed because copper is taken up by the enzymatic proteins not only at catalytically active centres, but also at inactive points from which the dialysis afterwards removes it. On ashing a water-dialysed enzyme, it was found that the ash had about 1/50 the catalytic activity of the enzyme itself, i.e. the enzymatic copper was 50 times as active as free Cu^{++} ion. Lovett-Janison and Nelson isolated from summer squash (*Cucurbita pepo* var. *condensa*) a copper-protein containing 0·15 per cent. Cu, giving greenish solutions, and having a high ascorbic oxidase activity; later preparations have had a higher percentage = 0·24, and shown activity proportional to their copper content.[4] Similar preparations from cucumber had about the same proportion of copper.[5] The enzyme has been crystallized.[6]

The dependence of the enzyme on copper is reflected in its susceptibility to inhibitors. It is reversibly inhibited 100 per cent. by M/1,000 HCN;[7] and also by M/1,000 H_2S.[8] It is also strongly inhibited by many chelating reagents forming copper complexes, such as 8-hydroxyquinoline, sodium diethyldithiocarbamate, and potassium ethylxanthate.[1] Inhibition may be almost complete with M/5,000 diethyldithiocarbamate,[2] but ascorbic oxidase from barley is not appreciably inhibited by carbon monoxide,[3] whereas polyphenolase is. The ascorbic enzyme has a fairly sharp pH optimum at about pH 6,[4] whereas polyphenolase may show little variation between pH 4·5 and 8·3. Hydrogen peroxide is apparently not formed, though it is said to be when the reaction is catalysed by free copper ions.[5]

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[1] Huszak, 1937; James and Cragg, 1943.

[2] Stotz, Harrer, Schulz, and King, 1938; Keilin and Hartree, 1938.

[3] A true ascorbic oxidase appears to be present in *Cucurbita maxima* (Tauber, Kleiner, and Mischkind, 1935); cauliflower (Hopkins and Morgan, 1936); *Moringa pterygosperma* (Srinivasan, 1936); cucumbers (Johnson and Zilva, 1937); apples (Hackney, 1946, 1949*b*); barley (James and Cragg, 1943); wheat (Waygood, 1950), &c.

[4] Powers, Lewis, and Dawson, 1944.

[5] Ramasarma, Datta, and Doctor, 1940; Stotz, 1940.

[6] Todokoro and Takasugi, 1939.

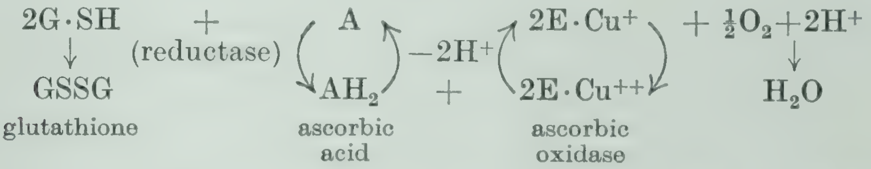
[7] Hopkins and Morgan, 1936; James and Cragg, 1943; Srinivasan, 1936.

[8] Srinivasan, 1936.

Ascorbic acid as carrier

It was first suggested by Szent-Györgyi that ascorbic acid might behave as a redox pair in plant respiration. At the time, mechanisms for the oxidation were known, but none for the reduction, and the idea hung fire.

The first cellular reductant to be discovered and investigated was glutathione.[6] It was shown that ascorbic oxidase itself is incapable of oxidizing the tripeptide, but that, in the presence of ascorbic acid and the enzyme from cauliflowers, there is continuous oxidation of glutathione, without loss of ascorbic acid until the glutathione is practically exhausted. In other words, the system



is in operation, with the ascorbic acid equilibrium lying far on the side of reduction. The rate of reduction was shown to be up to five times faster than the rate of oxidation, and to be catalysed by a second enzyme in the preparation. Direct reaction between glutathione and dehydroascorbic acid is slow. The dehydroascorbic reductase has been separated from the oxidase,[7] and found to be particularly abundant in cauliflowers, accounting for the maintenance of ascorbic acid almost wholly in the reduced form so long as glutathione is present. The reductase has been identified in 22 out of 30 plants examined[8] and occurs in varying amounts; where it is less abundant, as would be anticipated, there is perceptible loss of ascorbic acid in the presence of glutathione. Oxidized glutathione is believed to be reducible in plants,[1] but up to the present its reduction has not been attributed, directly or indirectly, to any glycolysis product.

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[1] King, 1939; James, 1946 and unpublished; James and Garton, 1952.

[2] James and Garton, 1952.

[3] James, unpublished.

[4] Srinivasan, 1936; James and Cragg, 1943; Tauber *et al.*, 1935; Waygood, 1950.

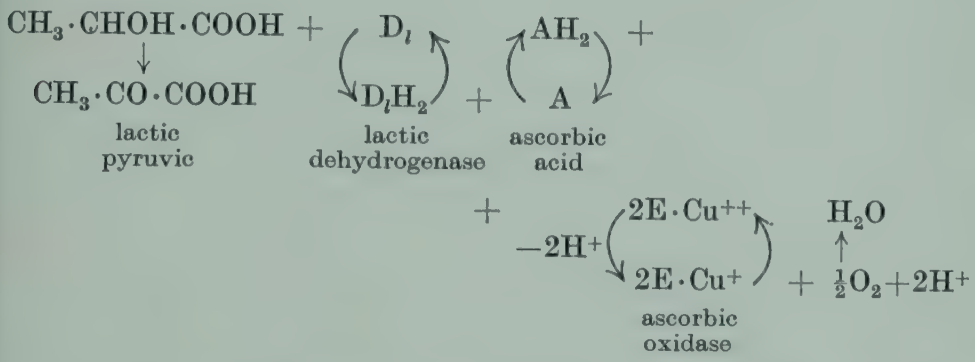
[5] Dawson, 1950.

[6] Hopkins and Morgan, 1936; Crook and Hopkins, 1938.

[7] Crook, 1941.

[8] Crook and Morgan, 1944.

Four systems linking with glycolysis have been assembled *in vitro* in which lactic acid, malic acid, triosephosphate, and alcohol respectively have been continuously oxidized by way of ascorbic acid. In the lactic acid chain,[2] set up with a barley extract, lactate is converted to pyruvate and reduced ascorbic acid maintained so long as lactate is present. In the absence of ascorbic acid there is no oxidation. Two enzymes are present, ascorbic oxidase and lactic dehydrogenase, the latter not requiring the addition of Coenzyme I. Other α-hydroxyacids, as glycollic and tartaric, are oxidized to a lesser extent. The system is probably to be written



Ascorbic oxidase has been separated from the dehydrogenase of such systems; but the dehydrogenase is fragile, readily lost during extraction, and destroyed by prolonged freezing.[3] It has therefore not been separated from the oxidase, indeed the difficulty is to preserve it even in a simply extracted system. Its separate existence in barley has been shown by the accelerated reduction of 2,6-dichlorophenolindophenol under nitrogen. A similar oxidation chain for lactic acid has been set up with wheat embryos, and Coenzyme I found to act as accelerator.[4]

The triosephosphate chain[5] co-operates with Coenzyme I. Hexosediphosphate added to clarified barley saps was converted to triosephosphate, and set up the sequence of oxidations. Loss of hexosediphosphate was related to oxygen consumption when ascorbic acid was added. A preparation dialysed for 42 hours still showed ascorbic oxidase activity, but little additional oxidation with hexosediphosphate. Addition of Coenzyme I caused uptake of oxygen much in excess of the equivalent of the coenzyme added; i.e. it catalysed the oxidation of endogenous substrates, and addition of hexosediphosphate enhanced this effect. Confirmation has been obtained with wheat preparations.[4]

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[1] Ibid.

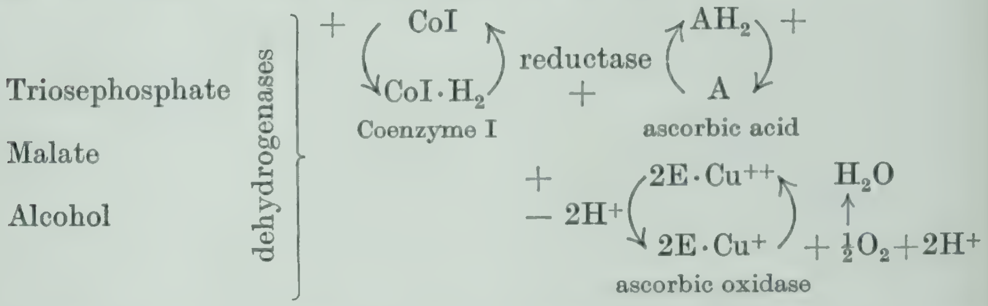
[2] James and Cragg, 1943.

[3] Beevers, 1947.

[4] Waygood, 1950.

[5] James, Heard, and James, 1944.

The malic acid and alcohol chains also operate through Coenzyme I.[1] Dehydroascorbic acid and Coenzyme I do not react directly,[2] so it may be presumed that a reductase exists in the barley and wheat preparations. The specificity of the dehydroascorbic reductase accepting hydrogen from glutathione has not been determined; but it is unlikely to work with Coenzyme I. An alternative possibility has been suggested by Waygood, who observed the existence of a redox pigment closely associated with ascorbic acid in wheat, and which was only in the visible, oxidized state when ascorbic acid concentration had been reduced to catalytic levels. These systems may be provisionally summarized:



The lactic and malic acid oxidations are inhibited by their α-keto-acid products. Addition of ketone fixatives or of glutamate, which in wheat saps transaminates the oxaloacetic acid formed from malic acid, allows the reaction to proceed.

Although the constituents have not yet all been obtained pure, the ability to synthesize these systems, with a fair knowledge of their effective contents, has shown at least some of the paths by which oxidation of glycolysis products is likely to go in wheat and barley.

THE FLAVOPROTEINS

*Iso*alloxazine forms nucleotides that have redox properties. The simplest is riboflavin (lactoflavin), a constituent of the B_2 group of vitamins, and widely distributed in plant tissues in concentrations within the range 1–10 μgm. per gm. tissue.[3] It is yellow and in water gives yellow solutions with a strong green fluorescence. It can be reduced by fairly strong reducers such as hydrosulphite (dithionite) with uptake of two atoms of hydrogen. Although commonly called a nucleotide for convenience and named after ribose, it contains the corresponding alcohol, ribitol, not the sugar itself. On reduction it becomes colourless and the fluorescence is quenched.

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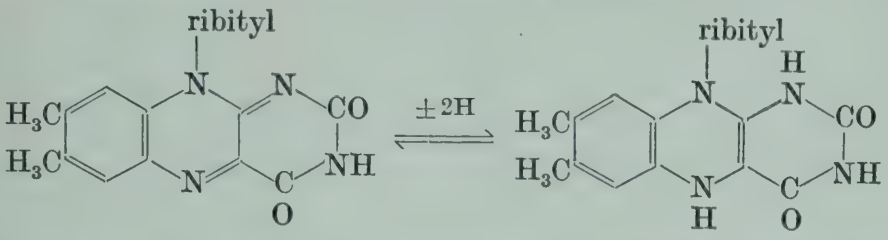
[1] Waygood, 1950.

[2] King, 1939.

[3] e.g. van Herk, 1935, pea seedlings; Euler and Dahl, 1935, oats; Copping, 1943, wheat; Barton-Wright and Booth, 1943, cereals and soybean; Bonner and Dorland, 1943, tomato plants; Kerly, 1944, tea and coffee.



Warburg and Christian (1932) isolated from yeast a 'yellow enzyme', which proved to have riboflavin phosphate as a prosthetic group. It was reduced by Dihydrocoenzymes I and II and reoxidized by molecular oxygen, though rather slowly under normal atmospheric conditions. It can be used to catalyse the transfer of hydrogen from reduced Coenzyme I to dyes such as methylene blue. It is usually now called the old yellow enzyme, others having been discovered since.

Following the same methods of extraction, van Herk (1937) found a flavoprotein of similar properties in the spadix of the aroid *Sauromatum*. He estimated the riboflavin present after conversion to lumiflavin, and found that almost all of it was linked with protein and non-dialysable. It was relatively abundant with a concentration of 8 μgm. per gm. fresh tissue. The spadix of *Arum maculatum* also contains a non-dialysable flavoprotein that has been extracted[1] into solution by a similar technique. It had the usual strong yellow colour and a pronounced green fluorescence. It was not reduced by ascorbic acid, but was easily reduced by hydrosulphite and, after such reduction, was autoxidizable, the colour and fluorescence returning rapidly upon shaking in air; and this cycle could be repeated many times. The prosthetic group of these aroid flavoproteins has not yet been obtained in sufficient quantity for identification.

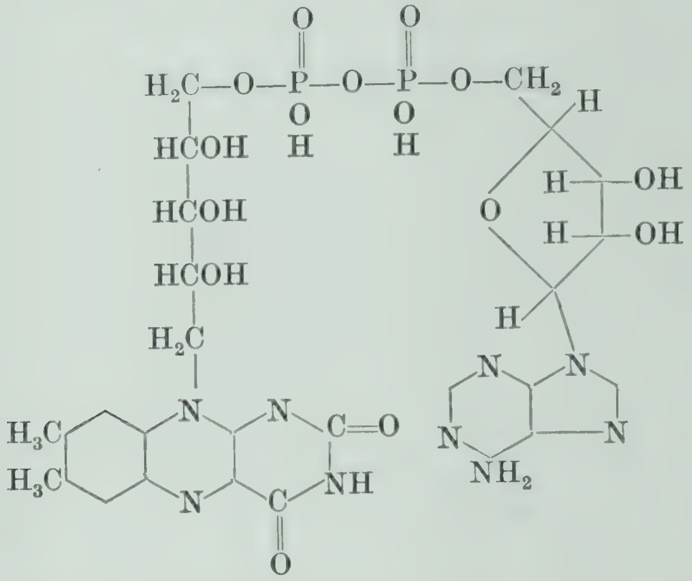
A second flavoprotein, having an *iso*alloxazine mononucleotide as prosthetic group, has been isolated from yeast.[2] It differs from the old yellow enzyme in reacting only with Coenzyme II and not with Coenzyme I, and in being virtually non-autoxidizable under physiological conditions. It vigorously reduces cytochrome *c*, and has been used to catalyse oxidation of hexose-6-phosphate by way of Coenzyme II and the cytochromes. It is accordingly named cytochrome reductase II.

The prosthetic group of other flavoproteins is an *iso*alloxazine adenine dinucleotide. The resemblance to Coenzyme I, a pyridine adenine dinucleotide, is evident, but the attachment of the *iso*alloxazine to the protein is much stronger, so that its dinucleotide behaves as a prosthetic group rather than as a coenzyme. It can be dissociated from

[1] James and Beevers, 1950.

[2] Haas, Harrer, and Hogness, 1942.

the protein and, providing the protein has not been injured by the treatment, the enzyme can be reconstituted by simple addition.



Diaphorase is a flavoprotein of this type which has been obtained from numerous tissues including seedling peas and beans.[1] It is specific for Coenzyme I and transfers hydrogen to redox dyes. It will not co-operate with cytochrome *c* as oxidant, and is not autoxidizable; its natural hydrogen acceptor is unknown. Solutions are yellow with a green fluorescence, and become colourless when reduced. The enzyme extracted from peas and beans was found to be very unstable in contrast with that from animal tissues, and has not been closely examined. Diaphorase is insoluble and is normally obtained in suspensions, but the more stable animal diaphorase can be brought into solution by warming to 43° C. in the presence of dilute ammonium sulphate and ethyl alcohol at pH 4·6.[2]

A complex, probably of flavoprotein nature, has been extracted from 6-day-old pea seedlings, and showed diaphorase activity towards Coenzymes I and II, i.e. it catalysed the transfer of hydrogen from both coenzymes to methylene blue. It further contained cytochrome *c* reductase transferring electrons from either coenzyme to the cytochrome.[3] Unlike the diaphorase previously obtained from pea seedlings, the preparations were stable.

Other flavoprotein enzymes include xanthine oxidase;[4] *d*-amino-acid oxidase,[1] the glucose oxidase of moulds, including notatin;[2] and probably the oxalic oxidase of the moss *Hyalocomium umbratum*.[3]

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[1] Lockhart, 1939. Small quantities of the dinucleotide itself have been found in clover, oats, potato, elder, runner bean, and chickweed by Whatley (1951*a*).

[2] Straub, 1939.

[3] Davison, 1950.

[4] Warburg and Christian, 1938*b*; Corran *et al.*, 1939.

The autoxidizable flavoproteins give rise to hydrogen peroxide when reacting with molecular oxygen. They have been classified as oxidases since they react with oxygen; and are sometimes called aerobic dehydrogenases to emphasize their difference from the metal-containing oxidases. Having no metallic prosthetic groups, they are not inhibited by low concentrations of HCN, H_2S, or sodium azide. No selective inhibitors for the *iso*alloxazine group are known.

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[1] Warburg and Christian, 1938*a*.

[2] Coulthard *et al.*, 1945.

[3] Franke and Hasse, 1937.

IX

OXIDATION STAGES IN RESPIRATION

TERMINAL OXIDASES OF PLANT TISSUES

SEVERAL systems have now been described that exist in plant tissues and which would appear capable, given the opportunity, of transferring electrons from glycolysis products to atmospheric oxygen. To decide whether, in any particular plant tissue, they do get the opportunity, that is to say whether they are playing a part in the tissue's respiration, is a further inquiry. Three methods of investigation exist and have, to varying extents, been exploited: (1) extraction and identification of the oxidases present in the tissue concerned, (2) enrichment of the tissue with a presumed or suspected carrier, and (3) application of specific inhibitors.

The first identifies the possibilities, and is usually the opening move of an investigation. The second has only a very limited scope, since carriers such as cytochrome *c* cannot be fed to a tissue from outside, or, like ascorbic acid, may already be present in saturating amounts. The onus falls mainly on the third method, the use of selective inhibitors, which again has its limitations due to lack of full specificity in the inhibitors, the non-existence of some much to be desired, and the resistance offered to entry of some of the reagents into living cells. It is also possible that enzymes combined in cells may be protected from combination with inhibitors with which they react in the free state. In spite of these difficulties, this remains the most effective available tool. The spectroscopic method that has been such a help in watching the cytochrome system at work in living yeast and insects is not available with many higher plants, on account of their low cytochrome concentrations and the absence of optical changes in the other carriers. A list of the principal respiratory inhibitors is given in Table 35. With a few tissues attempts have been made to assess the contribution of a particular system by means of its turnover number, that is to say by estimating the maximum rate of which it is capable. Conditions of reaction in and out of the cell are so different, and extractions likely to be so incomplete that it has not been possible to achieve much in this way.

A good deal of attention has recently been devoted to the problem of the terminal oxidases of plant respiration by several independent workers. As a result of their efforts it now seems tolerably certain that no single oxidase has a universal role, and that the important function of bringing oxygen into biological reaction is shared among the systems just described, and perhaps with others yet undetected. On account of the technical difficulties mentioned above, even the most careful observations must be interpreted with caution; but so much now seems well established. To suppose that any single oxidase was always present as the effective agent in all higher plant tissues would need drastic manipulation of available data.

Particular interest attaches to the role of cytochrome oxidase, since it is known to be a major respiratory agent in yeast and animal tissues. Good evidence of its playing a similar part in a number of higher plants is now to hand. Bhagvat and Hill[1] in addition to showing the presence of cytochrome and its oxidase in a considerable number of plant tissues (Table 34, p. 204), were able to demonstrate the redox behaviour of the cytochromes in favourable examples. Basal parts of onion bulbs soaked in water showed a gradual development of the absorption bands of reduced cytochromes; after removal of the water to allow free access of air, the bands disappeared again. In short, the cytochrome system was behaving as previously observed in yeast. Similar observations were made with young plumules of germinating runner beans, and have been confirmed with germinating barley embryos.[2]

Such experiments show that the cytochrome system is active in the tissues concerned, but do not indicate whether any significant uptake of oxygen results. At present this can only be achieved by the use of specific inhibitors. Respiratory oxygen consumption mediated by cytochrome oxidase would be expected to be inhibited by cyanide and azide. This would distinguish it from uptake by way of flavoprotein or other non-metallic systems; but not from uptake by way of copper enzymes such as polyphenol and ascorbic oxidases. The latter may be done by means of carbon monoxide, since only the cytochrome oxidase forms a carbon monoxide compound dissociable by light. The critical experiment is, therefore, treatment with carbon monoxide, with and without illumination.

Unfortunately, the inhibition is competitive and requires a high CO/O_2 ratio to be considerable. To reduce the oxygen uptake of *Torula* (yeast) to a half requires a ratio of about 20 to 1. All plant tissues so far examined show large variations of respiration rate with changes of oxygen pressure between 1·0 and 0·05 atm. It is therefore necessary to maintain the oxygen pressure at the same level in the presence of carbon monoxide as in the controls. To maintain total pressure at 1 atm. it is usual to use nitrogen as a filler, and comparisons are usually made between 95 per cent. CO with 5 per cent. oxygen and 95 per cent. N_2 with 5 per cent. oxygen.

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[1] Bhagvat, 1939; Bhagvat and Hill, 1951.

[2] James, unpublished.

TABLE 35

Respiratory inhibitors more or less specific in their action. Where no differentiating concentration-range is given, the inhibition is competitive

Reagent	*Inhibited*	*Vulnerable group*	*Not inhibited, or slightly inhibited*
M/1,000 sodium iodoacetate	phosphorylation (yeast)[1]		alcohol (animal)[6]; isocitric,[7] glucose,[8] and malic[4] dehydrogenases; fumarase,[4] zymohexase[37]
	triosephosphate dehydrogenase[2]	—SH	
	alcohol dehydrogenase (yeast[3] and plant[4])	—SH	
	glyoxalase[5]	—SH (glutathione)	
M/100 phloridzin	phosphorylation[9] (formation of glucose-1-phosphate[10])		phosphoglucomutase[10] zymohexase[11]
	triosephosphate dismutation[11]		amylase[9, 12]
M/100 sodium fluoride	phosphorylation[13]		phosphopyruvate → pyruvate + H_3PO_4[14]
	enolase[14]	Mg	isocitric dehydrogenase[7]
	cytochrome system[15]		glucose dehydrogenase[8] zymohexase[37]
1/1,000 aromatic sulphonic acids	carboxylase[16]	Mg	
1/5,000 malachite green	dehydrogenases[17]		polyphenol oxidase[18]
malonic acid	succinate ⇌ fumarate[19]		fumarase,[19] isocitric dehydrogenase[7]
M/10,000–M/1,000 arsenite	oxidative decarboxylation[20]		succinic oxidase (muscle),[21] alcohol dehydrogenase (yeast)[21]
trace H_2S	metal-containing oxidases[22]	Fe, Cu, &c.	flavoprotein oxidases[23]
M/10,000–M/1,000 HCN	ferric and cupric oxidases[24]	Fe^{+++} Cu^{++}	dehydrogenases[25] (except formic[26]), flavoprotein oxidases,[27] zymohexase[37]
M/1,000 sodium azide	ferric and cupric oxidases[28]	Fe^{+++} Cu^{++}	dehydrogenases, flavoprotein oxidases[29]
carbon monoxide	ferrous cytochrome oxidase (light reversible)[30]	haem	
	cuprous polyphenol oxidase (not reversible)[31, 32]	Cu^{+}	laccase,[32] ascorbic oxidase[33]
M/1,000 αα′-dipyridyl	zymohexase (yeast)[30]	Zn	zymohexase (muscle)[30]
M/1,000 *o*-phenanthroline	zymohexase (yeast)[30]	Zn	

TABLE 35 (*cont.*)

Reagent	*Inhibited*	*Vulnerable group*	*Not inhibited, or slightly inhibited*
M/1,000 8-hydroxyquinoline	ascorbic oxidase[34]	Cu	formic dehydrogenase[26]
M/5,000–M/1,000 potassium ethylxanthate	ascorbic oxidase[34, 35] cytochrome oxidase[35]	Cu Fe	
salicylaldoxime	polyphenol oxidase[31]	Cu	
M/5,000 sodium diethyldithiocarbamate	ascorbic oxidase[34, 35] polyphenol oxidase[35]	Cu Cu	cytochrome oxidase[35]
1/100,000 2,4-dinitrophenol	transfer of energy-rich phosphate[36]		

REFERENCES TO TABLE 35

[1] Beevers, 1950.
[2] M. Dixon, 1937; Meyerhof and Kiessling, 1935.
[3] M. Dixon, 1937.
[4] Berger and Avery, 1943*b*.
[5] See Turner, 1937.
[6] Lutwak-Mann, 1938.
[7] Berger and Avery, 1944.
[8] Ogura, 1939.
[9] Lundsgaard, 1933.
[10] Dahl, 1939.
[11] Kalckar, 1935.
[12] Barth, 1934.
[13] Tanko, 1936.
[14] Meyerhof, 1935*a*; Warburg and Christian, 1942.
[15] Borei, 1945.
[16] Axmacher and Ludwig, 1936.
[17] Quastel and Wheatley, 1931.
[18] James, Roberts, Beevers, and de Kock, 1948.
[19] Quastel and Whetham, 1925.
[20] Krebs, 1933.
[21] Barron *et al.*, 1947.
[22] Warburg, 1949; Keilin, 1928; Richter, 1934.
[23] Franke and Hasse, 1937.
[24] Warburg, 1949; Kastle and Loevenhart, 1901; Kubowitz, 1937*a*.
[25] Das, 1936; Euler, Adler, and Eriksen, 1937.
[26] Adler and Sreenivasaya, 1937.
[27] Franke and Hasse, 1937; Ogura, 1939.
[28] Warburg, 1949.
[29] Franke and Hasse, 1937.
[30] Warburg, 1949.
[31] Kubowitz, 1937*a*.
[32] Keilin and Mann, 1939.
[33] James, unpublished.
[34] King, 1939.
[35] James and Garton, 1952.
[36] Loomis and Lipmann, 1948; Teply, 1949.
[37] Allen, 1938. For further details about these inhibitors see James, 1953.

The terminal oxidases of barley embryos have been examined independently in England and America. Results of cyanide and azide inhibition are shown in Table 36.

TABLE 36

Percentage inhibition of O_2-consumption of barley embryos

	10^{-3} M HCN	10^{-3} M NaN_3	
15 hrs. from soaking .	82	78	Merry and Goddard, 1941
16 hrs. ,, ,, .	89	84	,, ,, ,,
17 hrs. ,, ,, .	88	86	,, ,, ,,
17 hrs. ,, ,, .	83	76	James and Garton, unpublished.

Both sets of investigators found strong inhibition by carbon monoxide which was light-reversible (Fig. 64 and Table 37).

TABLE 37

Inhibition of O_2-uptake by 95 per cent. CO+5 per cent. O_2 as compared with 95 per cent. N_2+5 per cent. O_2

	Inhibition per cent.	
Cytochrome oxidase		
Wheat embryos extract . . .	*c.* 80	Brown and Goddard, 1941
Barley ,, ,, . . .	72	James and Garton
Respiration (O_2-*consumption*)		
Wheat embryos	63 (79)	Brown and Goddard, 1941
Barley embryos 25° C. . . .	73 (91)	Merry and Goddard, 1941
,, ,, 30° C. . . .	56 (70)	James and Garton

Figures in parentheses give fraction due to cytochrome oxidase if oxidase inhibited 80 per cent. (Goddard) and 72 per cent. (James and Garton).

All the results with the three inhibitors are consistent with the hypothesis that the great bulk, up to 5/6, of the oxygen consumption of these cereal embryos is by way of cytochrome oxidase, the remaining sixth being due to some non-metallic system.

Somewhat similar results exist for carrot disks. The respiration of freshly cut disks, consisting partly of a wound respiration, is inhibited by M/1,000 cyanide or azide 70–80 per cent.[1] Under the conditions of experiment used by Turner and Hanly (1949), there was always a ground respiration of about 60 cu.mm. O_2/gm. F.W./hr., which was not inhibited by cyanide. After 200 hours in aerated distilled water, the cyanide-inhibited respiration had disappeared; but the ground respiration continued at about the same rate. Addition of succinate or KCl to the water induced a large increase of respiration, which was completely inhibited by M/1,000 cyanide. The isolation of a cyanide-stable fraction of the respiration is interesting, because it places beyond doubt the collateral activity of two types of oxidase in one tissue. Taken by itself, the survival of some 10–15 per cent. of oxygen uptake in the presence of M/1,000 cyanide, as often observed in plant tissues, might be open to other interpretations or even fall within limits of error. The accurate maintenance of low concentrations of cyanide in manometric experiments is difficult, and has become a matter of controversy.[1] The evidence of the carrot experiments seems, however, unequivocal. It has further been shown that the cyanide-sensitive component of the

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[1] Marsh and Goddard, 1939*a*.

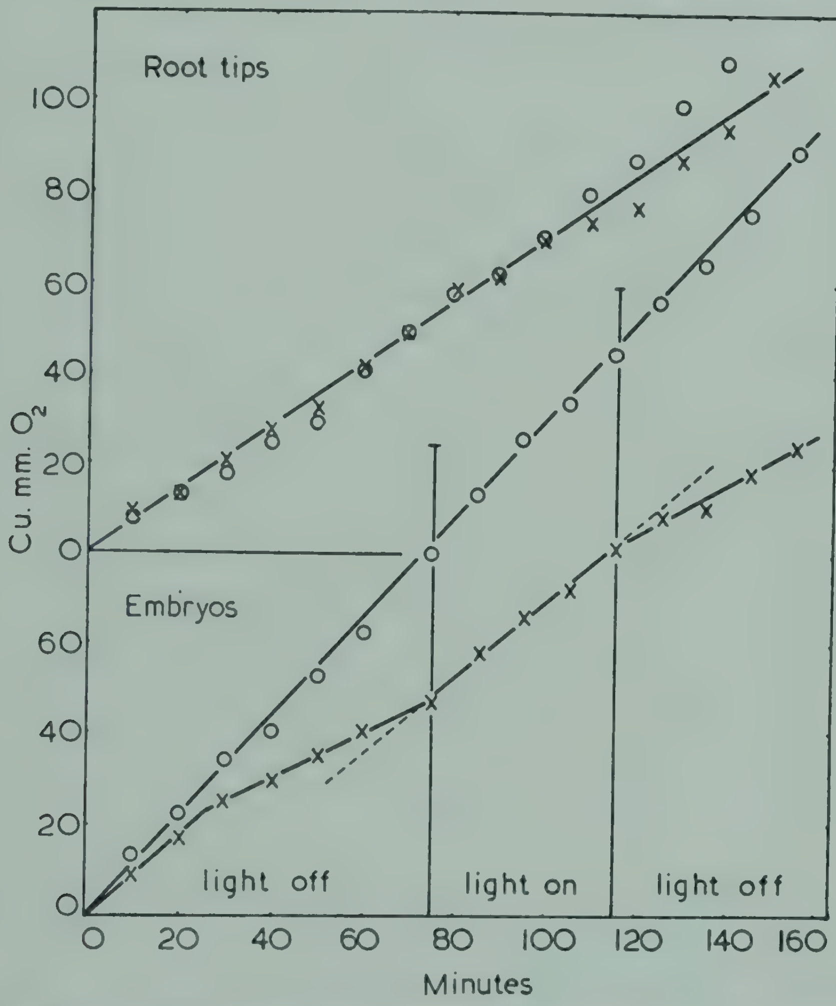

Fig. 64. Effect of 95 per cent. carbon monoxide on the oxygen uptake of barley embryos and root tips. James and Garton.

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[1] Krebs, 1935; Umbreit, Burris, and Stauffer, 1949; Laties, 1949*a*; James and Beevers, 1950.

respiration is also inhibited about 50 per cent.[1] or 66 per cent.[2] by 95 per cent. CO, and that this inhibition is reversed by light. Blue light is effective, but not red, in accordance with the known absorption spectrum of the cytochrome oxidase-CO compound (p. 203).

Light-reversible CO-inhibition of respiration has also been reported for a variety of other plant tissues, but there is considerable variation, and often a complete absence of inhibition (Table 38).

TABLE 38

Light-reversible inhibition of plant respiration by carbon monoxide

Inhibited	*Not inhibited*	
Young leaves of tobacco,* plum, oleander, pine	Mature white leaves of ivy, *Coleus*, *Zebrina*	Kempner, 1936
Young leaves, carrot	..	Marsh and Goddard, 1939*a*
Stamens, plum	Petals	Kempner, 1936
Pistils, daffodil	Roots	,, ,,
Pollen, *Lilium auratum*	..	Okunuki, 1939*a*
Germinating seeds, lupin	..	Tang, 1932
Young seedlings, wheat and rice	..	Taylor, 1942
Embryos, wheat	..	Brown and Goddard, 1941
,, barley	..	Merry and Goddard, 1941
	11-day root tips, barley	James, unpublished
Endosperm, barley	..	Merry and Goddard, 1941
	Spadix, *Arum maculatum*	James and Beevers, 1950
Root, carrot disks	..	Marsh and Goddard, 1939*a*; Weeks and Turner, 1950
Tubers, potato	..	Levy and Schade, 1948

* Photosynthesis narcotized by chloroform.

Cytochrome oxidase and cytochromes have been identified in most of the tissues showing the inhibition, and it is again noticeable that they are preponderantly embryonic or very young. Many of the older tissues examined were not CO-inhibited (Table 38), and did not yield cytochrome oxidase when treated by the usual methods.[3] Since negative results with inhibitors are so doubtful of interpretation, the positive demonstration of alternative systems in such tissues is highly desirable. This has been achieved with tea and *Arum* and the root-tips of barley seedlings.

So far as present evidence goes, cytochrome oxidase may be the universal terminal oxidase of plant embryos. In some tissues, such as carrot roots and barley endosperms, it appears to persist as the principal enzyme reacting with oxygen; but in others, such as barley roots and aroid spadices, it seems to be replaced partly or wholly by different systems.

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[1] Weeks and Robertson, 1950, using Danver's Half-long.

[2] Marsh and Goddard, 1939, using Nantes Coreless.

[3] James and Beevers, 1950, *Arum*; Sreerangachar, 1943, tea plant; Waygood, 1950, older wheat seedlings; James, unpublished, Mikhlin and Kolesnikov, 1947, barley seedlings.

The root tips of young barley seedlings react to inhibitors very differently from the embryos.[1] Ten-day-old tips, cut 1 cm. from the end, show little or no light-reversible CO-inhibition (Fig. 64). The participation of a metallo-enzyme is nevertheless indicated by almost total inhibition by cyanide and approximately the same by azide. There is, further, up to 62 per cent. inhibition by M/5,000 sodium diethyldithiocarbamate. This last reagent inhibits the respiration of embryos only slightly, and inhibits cytochrome oxidase extracted from the embryos by no more than 8 per cent.; but ascorbic oxidase is inhibited almost totally.[2]

Ascorbic oxidase is extractable from the root tips, but neither cytochrome oxidase nor polyphenol oxidase. It would therefore appear that the principal terminal oxidase of their respiration must be the ascorbic enzyme. The respiratory role of ascorbic oxidase in other tissues in which it is abundant, such as cucumber and cauliflower, does not yet seem to have been directly investigated.

The most difficult of the metallo-enzymes to assess in relation to respiration at the present time is polyphenol oxidase. The most acceptable evidence of its participation would be the occurrence of strong CO-inhibition not reversed by light. This does not yet seem to have been recorded for any plant tissue. Partial inhibition of spinach leaf respiration by M/1,000 *p*-nitrophenol[3] and of apple respiration by M/10 resorcinol[4] has been considered to indicate polyphenol oxidase activity. The former has, however, been shown[5] to inhibit the respiration of yeast which depends on cytochrome oxidase.

The polyphenol oxidase of potato tubers has for some time been considered the main terminal oxidase of their respiration; but it has now become common ground that part of this respiration proceeds through cytochrome oxidase.[6] The mean, light-reversible CO-inhibition observed by Levy and Schade was 35–40 per cent. If the potato cytochrome was inhibited to the same extent as the wheat enzyme (p. 220), this would mean that about half the respiration was catalysed by cytochrome oxidase. The experiments were necessarily carried out in 5 per cent. oxygen, so the estimate of inhibition applies to this concentration. Cytochrome oxidase activity is little reduced by a drop from air to 5 per cent. oxygen; but Levy and Schade showed that a second oxidase in potato homogenates was much more sensitive and, at 5 per cent. oxygen, showed only a quarter to a half of its aerobic rate of oxygen consumption. It is therefore clear that if, as they suppose, their second oxidase is responsible for the other part of potato respiration, it will be likely to account for a much larger proportion in air than in 5 per cent. O_2, and the cytochrome oxidase a correspondingly smaller proportion. Both enzymes are inhibited by cyanide, and perhaps the most interesting thing about these results is their strong suggestion that at least two metallo-enzymes must be operating as collateral terminal oxidases.

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[1] James, unpublished.

[2] James and Garton, 1952.

[3] Bonner and Wildman, 1946.

[4] Hackney, 1949*a*.

[5] Simon and Beevers, 1952.

[6] Levy and Schade, 1948; Schade and Levy, 1949; Boswell, 1950.

The identification of the second oxidase with polyphenol oxidase is a matter of dispute. Originally[1] it was suggested that polyphenol oxidase played a large part, mainly because suitable polyphenols, such as dopa, protocatechuic, caffeic and gallic acids, and an extract containing the natural potato polyphenols, raised the respiration of potato slices. The rise was long continued, the R.Q. remained at 1·0, the tissues were not browned, and the additional oxygen consumption was greatly in excess of the oxygen equivalent of the polyphenols added. There was thus every reason to suppose that they were behaving as redox carriers, accelerating electron transport from normal respiratory sources. Unfortunately, this does not necessarily indicate that the normal carriers, which they were supplementing, were of the same type as themselves. On existing data, it seems impossible to settle the point, and hence the exact significance of polyphenol oxidase in potato respiration. A fuller knowledge of its reaction to variations of oxygen pressure and to more specific inhibitors might provide the necessary information.

Non-metallic systems also play a part as terminal oxidases; frequently in association with one or other of the metallic systems already described. It has long been realized that the cyanide inhibition of many plant organs at differing concentrations of the inhibitor is incomplete and reversible. On removal of the external cyanide, inhibition is likely to disappear completely within an hour or so (Fig. 65). These observations are most readily explained by supposing that volatile HCN combines reversibly with the metal of the oxidase to form an inactive compound. The 'dissociation constant', $K = n/(1-n)[\mathrm{HCN}]$, where n denotes the uninhibited activity of the enzyme, can be calculated from the cyanide concentration and the amount of inhibition. In simple examples, this value remains reasonably constant over moderate ranges of cyanide concentration and, in the words of Warburg (1949), "the question as to how complete is the inhibition by cyanide is answered by

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[1] Boswell and Whiting, 1938; Baker and Nelson, 1943; Boswell, 1945; Nelson and Dawson, 1944.

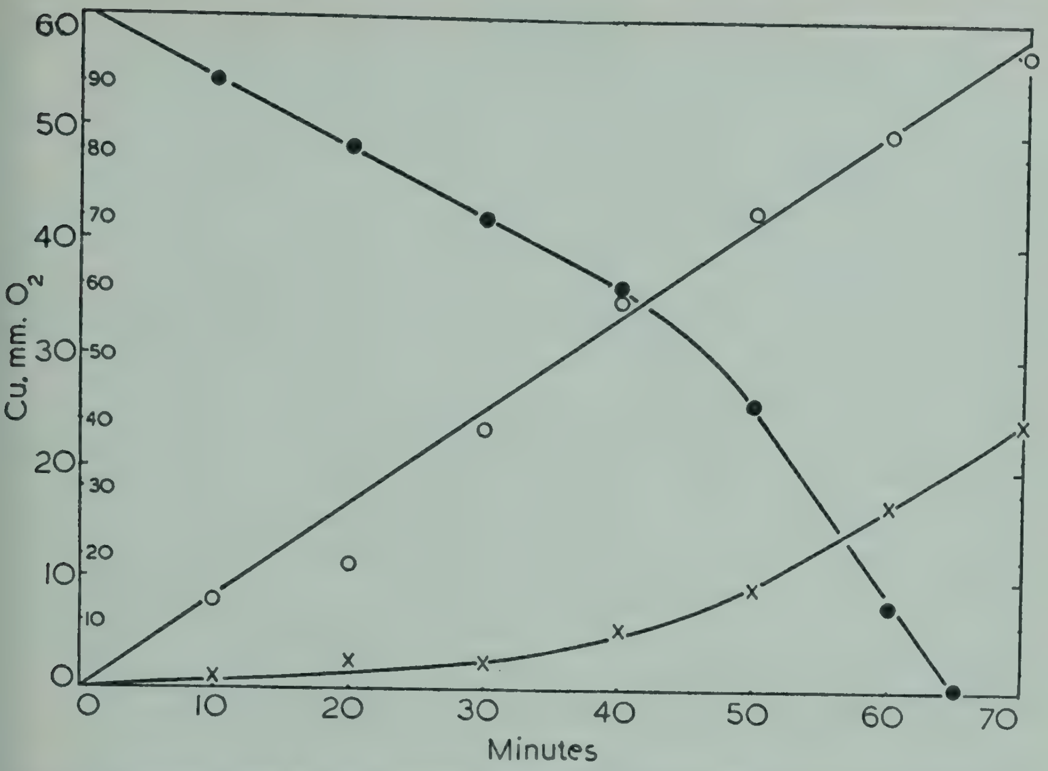

FIG. 65. Inhibition by M/1,000 cyanide. Oxygen uptake by 50 root tips of barley. ○, uninhibited; ×, tips inhibited by prolonged treatment with M/1,000 cyanide which was removed immediately before the measurements began; ●, percentage inhibition on scale given by small figures. Inhibition at first was total, and was completely reversed in about 65 minutes.

the statement that it is as complete as is required by the dissociation law'.

The explanation itself is not, however, complete, at least when applied to plant tissues; the range of inhibition shown is too great (Table 39). The inhibition of the tissue respiration is often less than that of the extracted oxidase. For example, cytochrome oxidase is completely inhibited by M/1,000 HCN, but wheat and barley embryos and carrot disks, in all of which it is known to be active, are inhibited only 70–92 per cent. Results with M/1,000 sodium azide show a similarity to those with cyanide[1] but with certain differences. At neutral or mildly acid pH, hydrogen cyanide is virtually undissociated; at pH 7·5 and 25° C. the dissociation constant, $K = 0{\cdot}022$. Hydrazoic acid, on the other hand, is already half dissociated at pH 4·7. Whereas change of pH in mildly acid regions has little effect on cyanide poisoning, the effect on azide poisoning is very marked. At pH 4·5, M/1,000 azide inhibits the oxygen consumption of young and full-grown carrot leaves about 80 per cent.; but at neutral reactions the older leaves show a

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[1] Marsh and Goddard, 1939*a*; Merry and Goddard, 1941; Morrison, 1949.

TABLE 39

Percentage inhibition of O_2-uptake by some plant tissues in the presence of M/1,000 *cyanide*

Oat, coleoptiles—76 hrs.	74	Bottelier, 1939
96 hrs.	57	,, ,,
Wheat, embryos	92	Brown and Goddard, 1941
root tips 0–3 cm.	65	Lundegårdh, 1949
root, zone 3–6 cm.	44	,, ,,
Barley, embryos	82–89	Merry and Goddard, 1941; James, unpublished
endosperm	80	Merry and Goddard, 1941
root tips	100	James, unpublished
Tomato, root tips	39	Henderson and Stauffer, 1944
Carrot, root disks	70–74	Marsh and Goddard, 1939*a*
young leaves	61–72	,, ,, ,,
old leaves	nil or increase	,, ,, ,,
Rhubarb leaves	75	Morrison, 1949
Tea leaves	35	Li and Bonner, 1947
Spinach leaves	49	Bonner and Wildman, 1946
Arum spadix	0–10	James and Beevers, 1950
Chlorella	73	Genevois, 1929*b*
Aspergillus, submerged hyphae	80	Tamija, 1942
surface hyphae	14	,, ,,

stimulation.[1] The same effect is observed with full-grown barley leaves. Similar concentrations of cyanide cause inhibition and no stimulation,[2] and in acid solutions, where M/1,000 azide appears to be acting purely as an inhibitor, the inhibition is quantitatively similar to that of cyanide and is also reversible. At neutral pH, oxygen consumption is again slightly stimulated.

Various explanations might be offered: the enzyme *in situ* might be less sensitive than when extracted; the inhibitor might fail to penetrate the tissue; or, most important, a second enzyme of much higher resistance to cyanide poisoning, i.e. an enzyme not depending upon a metal for its oxidase activity, might be acting collaterally. It is with the last possibility that we are concerned. Since the survival of 10–20 per cent. respiration in M/1,000 cyanide is ambiguous taken in itself, it is fortunate that the resistant fraction can be separated in other ways. The ground respiration of carrot disks, which survives 200 hours of aerated washing, is wholly unaffected by M/1,000 cyanide and approximately equal in rate to the initial cyanide-resistant fraction (p. 220). The autotrophic respiration of *Chlorella* is also highly resistant to cyanide poisoning,[3] though the respiration of added sugars is inhibited at the usual cyanide concentrations.[4]

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[1] Marsh and Goddard, 1939*a*; Stenlid, 1949*a*.

[2] James and Hora, 1940.

[3] Warburg, 1949.

[4] Emerson, 1927; see Commoner, 1940, for a general review.

The residual cyanide-resistant respiration in such materials is always slow with Q_{O_2} of the order of 10^{-2}. The nature of the terminal oxidase concerned is uncertain. A much faster respiration with $Q_{O_2} > 30$ occurs in the spadix of aroids during the unfolding of the inflorescence, and is practically unaffected by cyanide even at relatively high concentrations. During the opening of the spathe of *Arum maculatum*, when the respiration rate is at its fastest, inhibition by M/1,000 HCN is nil. There is no inhibition by M/500 cyanide in the spadices of the related *Arum creticum*, *Arum italicum*, *Aerissima amurense*, *Amorphophallus rivieri*, and *Biarum tenuifolium*. There is also no inhibition by carbon monoxide or M/1,000 diethyldithiocarbamate. It is not possible to extract cytochrome, polyphenol, or ascorbic oxidase; but cell-free extracts contain a highly active oxidase of low oxygen affinity, showing increasing rates of oxygen uptake even at 100 per cent. O_2 (1 atm. pressure). Addition of riboflavin, with or without adenosinetriphosphate, increased its activity. Further purification of an extract from *Arum maculatum* spadix has yielded a solution containing the properties of an autoxidizable flavoprotein.[1] Van Herk (1937) found unusually large amounts of flavoprotein in *Sauromatum* spadix by extraction and estimation as lumiflavin.

It thus seems clear that no metallo-enzyme is acting as terminal oxidase to spadix tissue. Specific inhibitors for autoxidizable flavoproteins are not known; but the predominant role of such an enzyme in spadix respiration is inferred from the following facts. No other type of oxidase has yet been extracted; oxygen consumption by the tissue is also unaffected by M/1,000 HCN, M/1,000 diethyldithiocarbamate, and 95 per cent. carbon monoxide; and has an exceptionally low 'oxygen affinity' in agreement with the known properties of flavoproteins[2] and the crude enzyme extract. Riboflavin, the prosthetic group of some flavoproteins, co-operates vigorously in the oxygen uptake of cell-free preparations.

Surveying the data now available, we may conclude, perhaps, that the cytochrome system occupies a special position in the transport of electrons to oxygen by higher plant tissues. Cytochrome oxidase appears to be the dominant oxidase in embryonic tissues of seeds and very young leaves, and in the induced respiration, often associated with cambial activity, at the cut surfaces of storage tissues. It has also been reported in endosperm, a tissue of brief development, and in active organs of reproduction.

Even in these young or regenerated tissues, it is rarely the only terminal oxidase at work. It is almost always associated with a cyanide-resistant partner, and sometimes apparently with other metallo-enzymes also. Owing to the heterogeneity of the tissues used, it is not always clear that both enzymes are present in a single cell, but there is no evidence of segregation. Quantitatively, cytochrome oxidase appears to be the most effective oxidase in meristematic tissues, and to be responsible for by far the largest part of the oxygen consumption.

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[1] James and Beevers, 1950.

[2] Laser, 1952.

Satisfactory evidence of the activity of cytochrome oxidase in the respiration of adult plant tissues is much rarer, and evidence of its absence or non-participation is commoner (Table 38, p. 222). The rate of respiration also becomes slower; but the disappearance of the cytochrome system is accompanied by the appearance of new systems in its place. One of the most striking examples is that described in barley seedlings. The soaked embryos depend on the cytochrome system more than 80 per cent., with a residual metal-free component; but in root tips, after 10 days' development, cytochrome activity is not detectable. The principal agent is then ascorbic oxidase with possible assistance from an unidentified metallo-enzyme; there is little or possibly no cyanide-resistant fraction.

While it seems certain that various terminal oxidases, and not one only, are normally functional in plant tissues, it is not yet possible to assess the relative importance of different systems, or how extensively the cytochrome system is replaced by others in natural tissues. Since the early attempt by Onslow (1931), little has been achieved in studying the systematic distribution of the plant oxidases, and virtually nothing concerning their actual functioning. It may prove that certain families

TABLE 40

Tissue	*Oxidases*	
	Acting	*Not acting*
BARLEY		
Embryo	cytochrome $+n$	ascorbic
Young root tips . . .	ascorbic $+m$	cytochrome
Young leaves . . .	cytochrome	polyphenol
CARROT		
Young leaves . . .	cytochrome $+n$	. .
Root disks	cytochrome $+n$	polyphenol
POTATO		
Tuber disks . . .	cytochrome $+m$ polyphenol ?	. . . .
AROIDS		
Spadix	flavoprotein	cytochrome, ascorbic polyphenol
Stalk.	'polyphenol'	cytochrome, ascorbic

m = unidentified enzyme inhibited by cyanide.
n = unidentified enzyme not inhibited by cyanide.
References in the text.

tend to employ a particular oxidase; but it is yet too early to say so. In Table 40 are collected the probabilities for a few of the most investigated tissues. It serves to indicate the apparent predominance, falling short of universality, of the cytochrome system. It is possible that, when the respiration of adult tissues has been more studied, this predominance may be less marked.

THE LINKS BETWEEN GLYCOLYSIS AND OXIDATION

Since the final products of plant glycolysis, such as alcohol and lactic acid, do not appear under aerobic conditions, interruption of the reaction sequence must occur at one or more intermediate stages. Alcohol itself is not readily oxidized by many plant tissues and the probability now is that oxidation of intermediate products occurs in a series of stages providing a number of different substrates for the oxidation systems. Members of the glycolysis sequence liable to serve are hexosemonophosphates, triosephosphate, pyruvic acid, and acetaldehyde. Triosephosphate occupies a special position because it is oxidized to phosphoglycerate even during anaerobic glycolysis. Its aerobic oxidation does not, therefore, imply any deviation of the main reaction chain; but only a diversion of electrons to a new acceptor, oxygen, in place of the internal acceptor, acetaldehyde. The oxidation of any of the other substances mentioned would cause the formation of a new product, and set up a new reaction sequence.

Identification of the loci of the oxygen-operated switch has been attempted by the use of inhibitors, especially iodoacetate. Unfortunately, the effects of iodoacetate in enzymatic systems are not too clear. The known effects are summarized in Table 35, p. 218; but not all of them necessarily apply to the higher plants. Contrary to an early expressed view,[1] M/1,000 iodoacetate does strongly inhibit plant respiration, though it may take a relatively long time for the full effect to develop. Turner (1937) showed that the time taken is longer in air than in nitrogen, and that an apparent failure to poison aerobic respiration of carrot disks is reversed after 7 or 8 hours. Inhibition of respiration by dilute iodoacetate has now been shown in, for example, tomato stems[2] and roots,[3] barley roots[4] and spinach leaves,[5] oat coleoptiles,[6] pea internodes,[7] and *Arum* spadix.[8] Inhibition by dilute sodium fluoride has been shown to occur in a similar range of tissues.[2, 4–8] Iodoacetate is known to inhibit triosephosphate dehydrogenase, and fluoride to inhibit enolase, which converts phosphoglycerate to phosphopyruvate. Both substances also interfere with phosphorylation (Table 35) and possibly with other reactions as well. Unfortunately, the intermediate substances accumulating in the poisoned tissues have not yet been identified. During the breakdown of hexosediphosphate in barley saps, iodoacetate poisoning leads, as would be expected, to the accumulation of triosephosphate; and fluoride poisoning leads to accumulation of an ester resembling phosphoglycerate.[1] It may well be that the action of these inhibitors results from the normal course of respiration passing through phosphoglycerate and phosphopyruvate, even in the presence of air; but the steps needed to make this evidence conclusive have not yet been taken.

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[1] Boysen-Jensen, 1931.

[2] Caldwell and Meiklejohn, 1937*b*.

[3] Henderson and Stauffer, 1944.

[4] Machlis, 1944*a*; Laties, 1949*b*.

[5] Bonner and Wildman, 1946.

[6] Bonner, 1948.

[7] Christiansen and Thimann, 1950.

[8] James and Beevers, 1950.

At the next stage, formation of pyruvate, the data are more compelling. Aromatic sulphonic acids poison carboxylase, whose substrate is pyruvic acid. The fact that the specificity of these inhibitors is not known in detail is of minor importance, because it has been shown that 1-naphthol-2-sulphonic-acid does cause the accumulation of pyruvic acid in barley leaves respiring in air.[2] The use of sodium fluoride stops pyruvate formation,[3] and it is, further, significant that the addition of M/20 pyruvate to detached barley roots lifts their rate of respiration in the presence of fluoride or iodoacetate back virtually to the normal.[4] The respiration of uninhibited barley embryos and young leaves is also much increased by pyruvate-feeding, with a rise of R.Q. towards the pyruvate value, 1·2.[5]

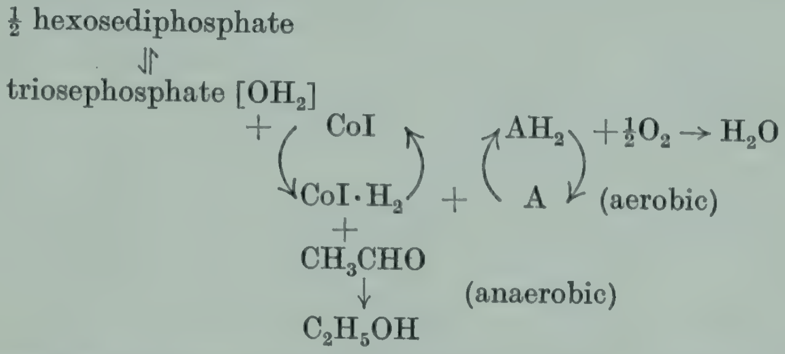
It seems safe to conclude that plant glycolysis proceeds in air at least as far as pyruvic acid; but this is not inconsistent with the occurrence of oxidations at earlier stages, especially of triosephosphate. Carboxylase is not inhibited in air,[5] and traces of acetaldehyde have sometimes been obtained from aerobic tissues (p. 136); but attempts to study the oxidation of acetaldehyde in plant tissues have so far met with little success.[6] Attention has mainly been concentrated on triosephosphate and pyruvic acid. The oxidation of lactic and other α-hydroxy-acids that may arise glycolytically has also been analysed.

Triosephosphate

Mechanism for the aerobic oxidation of triosephosphate has been shown to exist, and it takes the form of a switch of electrons from acetaldehyde to a respiratory carrier. In barley saps, the carrier role

[1] James, Heard, and James, 1944.
[2] G. M. James and James, 1940.
[3] James, James, and Bunting, 1941.
[4] Laties 1949*b*; see also Bonner and Wildman, 1946 (spinach leaves).
[5] James and Norval, 1938.
[6] Goddard and Meeuse, 1950.

is played by ascorbic acid; and the reactions, omitting enzymes, may be outlined



Dehydroascorbic acid competes successfully with acetaldehyde for the hydrogen of reduced Coenzyme I, and alcohol formation is extinguished. A similar system has been extracted from wheat (p. 211), and an analogous one from the pollen of *Lilium auratum* in which cytochrome replaces the ascorbic acid.[1] In both systems a reductase catalyses the transfer of hydrogen from the reduced Coenzyme I to the carrier.

The characteristics of the respiration of young barley tissues and lily pollen agree with the supposition that they largely depend on the ascorbic and cytochrome systems respectively. The barley respiration is heavily inhibited by diethyldithiocarbamate and does not show light-reversible CO-inhibition,[2] which does occur in lily pollen.[1]

α-hydroxyacids

These are oxidized by the ascorbic system in barley and wheat saps. Lactic acid is most vigorously oxidized by barley preparations and does not need addition of Coenzyme I. Glycollic and tartaric acids are also oxidized. A similar oxidation of lactic acid in wheat seedlings is accelerated by Coenzyme I (p. 211). Lactic dehydrogenase has been extracted from lily pollen, and, in association with its accompanying cytochrome oxidase and added cytochrome *c*, sets up a continuous oxidation system.[1] Glycollic acid is also oxidized by an enzyme which does not contain a heavy metal, and does not co-operate with a carrier. The enzyme has been extracted from numerous green plant organs.[3] Labelled glycollic acid synthesized by many tissues in light is rapidly consumed in the dark.[4] Malic acid is one of the most widely discovered acids in plants. Its oxidation has been carefully studied in wheat preparations, in which it goes by way of the ascorbic system (p. 212). All these acids might conceivably act as links between glycolysis and oxidation; but their respiratory status is at present still uncertain.

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[1] Okunuki, 1940.

[2] James, unpublished.

[3] Clagett, Tolbert, and Burris, 1949.

[4] Benson and Calvin, 1950.

Pyruvic acid

Pyruvic acid has for a considerable time been recognized as a nodal point of metabolism in plants, as in other organisms. As the simplest of the α-ketonic acids, it embodies numerous possibilities of reaction of which the following are realized biologically:

TABLE 41

Some biologically catalysed reactions of pyruvic acid

1. Decarboxylation . .	$\rightarrow CH_3CHO + CO_2$	yeast, plants
2. Oxidative decarboxylation	$\rightarrow CH_3COOH + CO_2$	animals, bacteria
3. Hydrolysis . . .	$\rightarrow CH_3COOH + H{\cdot}COOH$	bacteria
4. Dismutation . . .	$\rightleftharpoons CH_3COOH + CO_2 +$ lactic acid	Gonococci
5. Reduction . . .	$\rightleftharpoons$ lactic acid	yeast, animals, plants
6. Amination . . .	$\rightleftharpoons$ alanine	many tissues
7. β-carboxylation . .	$\rightleftharpoons$ oxaloacetic acid	liver
8. +oxaloacetic acid . .	$\rightleftharpoons$ citric acid, &c.	muscle
9. Glycolysis (reverse) . .	$\rightleftharpoons$ carbohydrates	many tissues ?

Through glycolysis, pyruvic acid is associated with carbohydrate formation, through amination with protein synthesis, and through acetic acid with fat metabolism. Reactions such as 2 and 8 are likely to be significant in leading to energy transformations in oxidation.

The tricarboxylic acid (Krebs) cycle

The difficulty of elucidating the further oxidation of acetic acid led to searches for alternative methods of pyruvic degradation. A most significant observation was made by Krebs and Johnson (1937) who, incubating minced muscle anaerobically with added pyruvate and oxaloacetate, proved the formation of substantial amounts of citric acid. In air, similar preparations poisoned with malonate, formed succinic acid. Since oxaloacetate may be again formed when these acids are degraded, it can react with further pyruvate; in short, catalyse pyruvate decomposition. Each time a molecule of oxaloacetate and pyruvate react, a cycle of reactions is performed in which the oxaloacetate is remade and the pyruvic acid decomposed to its equivalent CO_2 and water. The cycle is complex, and citric acid may be a side arm, not a direct constituent of it.[1] The cycle as it is at present supposed to occur in animal tissues goes as in Fig. 66.[2]

The first reaction is an oxidative decarboxylation probably akin to that of bacteria, in which inorganic phosphate and numerous other catalysts in addition lead to the intermediary formation of acetyl phosphate. What precise substance actually reacts with oxaloacetic acid in reaction 2 is still an open question. Reaction 6 is also an oxidative decarboxylation and, like reaction 1, is virtually irreversible. As a result, although the other partial reactions are readily reversible, the cycle as a whole is not. Reaction 6 is strongly inhibited by M/1,000 arsenite, and when this is added to minced liver supplied with pyruvate, α-ketoglutarate accumulates. Another important inhibitor of the cycle is malonate, which competes with succinic acid for the succinic dehydrogenase catalysing reaction 7; its presence leads to the accumulation of succinic acid. Under anaerobic conditions, when the later oxidative stages are repressed, citric acid appears. This is due to the presence of the enzyme aconitase, which catalyses the hydration reactions 3 and 10. The apparent oxidation of citric acid is also due to the same enzyme, converting it to *iso*-citric acid. It is the latter that is really dehydrogenated, by an *iso*-citric dehydrogenase requiring Coenzyme II and inactive with Coenzyme I. The hydration reaction 8 is catalysed by the enzyme fumarase.

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[1] Krebs, 1942; but other authors retain citric acid in the direct cycle.

[2] For recent summaries of the evidence from animal sources see Baldwin, 1952; H. G. Wood, 1946.

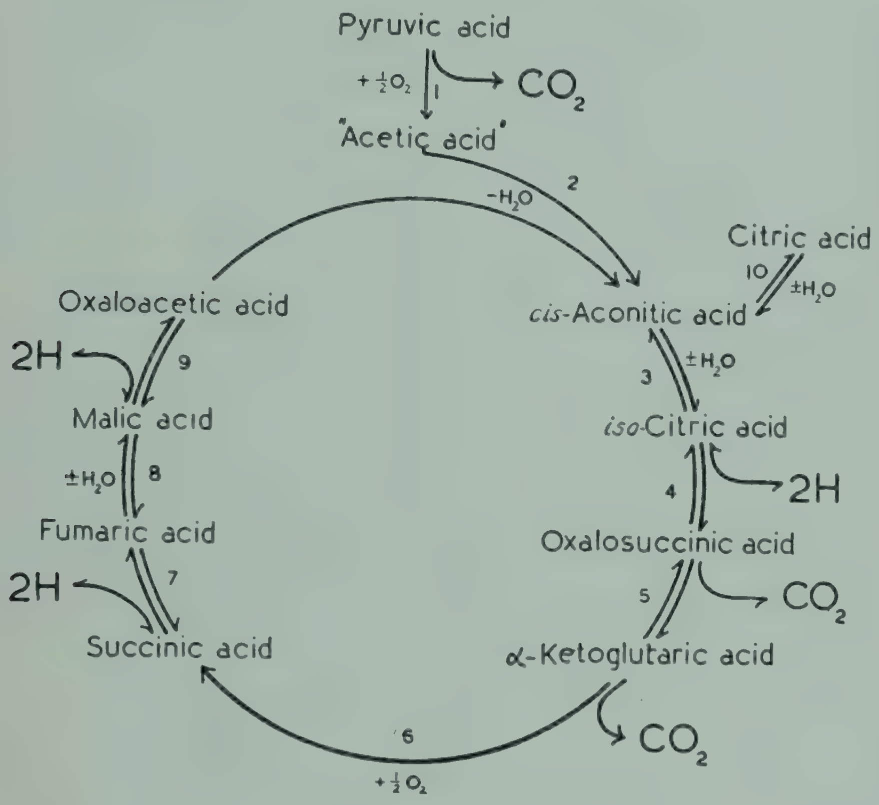
Pyruvic acid
$+\frac{1}{2}O_2$
1
CO_2
'Acetic acid'
2
$-H_2O$
Citric acid
10
$\pm H_2O$
cis-Aconitic acid
3
$\pm H_2O$
iso-Citric acid
4
2H
Oxalosuccinic acid
5
CO_2
α-Ketoglutaric acid
CO_2
6
$+\frac{1}{2}O_2$
Succinic acid
7
2H
Fumaric acid
8
$\pm H_2O$
Malic acid
9
2H
Oxaloacetic acid

FIG. 66. The tricarboxylic acid cycle.

The full importance of the cycle to animal respiration is underlined by the fact that small additions of its members catalyse large increases of respiration in which the R.Q. is unaltered. This was first demonstrated, even before the full cycle was developed, by Szent Györgyi[1] with succinate and fumarate in minces of pigeon-breast muscle, which respires rapidly and produces little or no lactate. Extensive reinvestigation confirmed his results. The ground muscle respires very rapidly at first, the rate slowly dropping away as oxaloacetate, the unstable substance, is lost. Stare and Baumann (1937) showed that addition of small amounts of fumarate maintained or restored the original rate of oxygen consumption, malonate inhibited it, and mixtures of malonate and fumarate were as effective as fumarate alone. It was shown by direct estimation that the added fumarate was not used up, and that the additional oxygen consumption was 6–10 times the amount required for the complete oxidation of the amount of fumarate added; in other words that the fumarate was behaving catalytically. Small additions of succinate, oxaloacetate, and malate gave similar increases of oxygen uptake without alteration of the R.Q.; large additions caused a rise of R.Q., indicating that excess fumarate, &c., were consumed without replacement. Shortly after, it was shown that citrate and α-ketoglutarate caused similar increases of oxygen uptake,[2] and that fumarate in the presence of excess pyruvate, which hindered the further oxidation of α-ketoglutarate, caused accumulation of the last. An important point was also made about malonate poisoning. Since only succinic acid was found to accumulate in its presence, it was presumed to act specifically on succindehydrogenase. The action is also competitive, depending on the ratio malonate/succinate. When this was about 9·5, inhibition was 50 per cent. and, since internal concentrations of succinate are always very low, M/1,000 malonate may be expected to cause high percentage inhibitions. Very low concentrations of malonate will thus allow the cycle to proceed at a reduced rate, especially if the succinate concentration is artificially increased, either by addition of succinate itself or one of its known precursors. High concentrations of malonate, on the other hand, will block the cycle more or less completely, and bring oxygen consumption to a virtual standstill when all intermediates have been converted to succinate. These expectations were realized in the experiments of Krebs and Eggleston with preparations of pigeon-breast muscle.

If the respiration of a tissue is proceeding to any considerable extent through the tricarboxylic acid cycle, these criteria will be satisfied:

1. The intermediate substances will be present, or detectable after suitable treatment of the tissue.
2. The appropriate enzymes will also occur.

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[1] Laki, Straub, and Szent Györgyi, 1937.

[2] Krebs and Eggleston, 1940.

3. Small additions of intermediates will cause relatively large increases of oxygen consumption without alteration of the R.Q. Large additions may overload the machinery, so that there is oxidation of the substance added without replacement, and a consequent rise of R.Q. Small additions will be maintained by the operation of the cycle, provided that adequate pyruvate is forthcoming from glycolysis or by simultaneous addition.

4. Moderate additions of malonate, about M/40, will, after a brief induction period, bring oxygen consumption virtually to a standstill.

5. Succinate will be formed oxidatively from pyruvate+fumarate, even when its reductive formation from fumarate is precluded by malonate poisoning.

6. Malate, oxaloacetate, α-ketoglutarate, and other intermediates will also lead to succinate formation under similar conditions.

7. Other inhibitors such as arsenite, excess pyruvate, and anaerobic conditions will cause the accumulation of the appropriate intermediates.

No one of these points taken singly can demonstrate the existence of the cycle, since each might have an alternative explanation. In particular, it needs emphasis that malonate poisoning of oxygen uptake only demonstrates the activity of succindehydrogenase, and not, by implication, the existence of the whole cycle. Szent Györgyi originally suggested that succinate $\leftrightharpoons$ fumarate$+2$H, catalysed by succindehydrogenase, might act as redox system accepting 2H from glycolysis intermediates; and it has been supposed that this occurs in muscle during triosephosphate oxidation, which is admittedly outside the scope of the acid cycle.[1] Whether this happens or not, it cannot be assumed without further proof that succindehydrogenase has no existence apart from the cycle.

A sufficient body of proof has been built up for animal tissues to obtain acceptance for the tricarboxylic acid cycle as a major pathway of oxidation. There is much less unanimity of opinion in regard to bacteria, yeast,[2] and the higher plants. There follows a summary of the evidence available from plants, arranged according to the criteria listed.

1 and 2. Acids and enzymes of the tricarboxylic acid cycle in plants

Nearly all the acids of the cycle have been found, more or less widely distributed in the higher plants. *Cis*-aconitic acid has been reported in the juice of sugar canes;[3] but Krebs and Eggleston (1944) were unable to detect it in the leaves of any one of 18 species. The enzyme aconitase, converting *cis*-aconitic acid into citric or *iso*-citric acid, has been identified in chickweed,[1] oat coleoptiles,[2] and potatoes.[3]

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[1] Krebs and Eggleston, 1940.

[2] See, e.g. Krebs, 1949; Foulkes, 1951; Krebs, Gurin, and Eggleston, 1952.

[3] Cited from Thimann and Bonner, 1950.

Pucher and Vickery demonstrated in 1942 that much of the 'malic acid' of crassulacean succulents was in reality *iso*-citric acid, and this was shortly afterwards confirmed by Krebs and Eggleston (1944). Considerable amounts, up to about 13 per cent. of the dry weight, were found in the leaves of *Sedum* and *Bryophyllum* species. Foxglove leaves contained 1·93 per cent.; but none could be found in the leaves of 11 other diverse species. *Iso*-citric dehydrogenase is probably of wide occurrence (Table 30, p. 187). Oxalosuccinic and α-ketoglutaric acids have not yet been identified in plants. A method developed by Pucher and Vickery in 1941 revealed the presence of succinic acid in tobacco leaves, and has since led to its identification in a number of other species.[4] Succinic dehydrogenase has been recognized in a considerable number of plants (Table 30, p. 186); but has been reported absent from others. A survey of the seedlings of 16 Indian species showed it to be present in 8 and apparently absent from 8. It was demonstrable in young seedlings of *Phaseolus mungo*, but absent from mature shoots,[5] an interesting point in view of its close association with the cytochrome system. Negative results may be due to the presence of an inhibitor which gains access to the enzyme during extraction;[6] but this has not been shown to apply to all apparently deficient species. Fumaric acid has been identified in tobacco leaves,[7] and fumarase in oat coleoptiles.[8]

Malic acid has a very wide distribution in plants and is found in relatively large amounts. It is particularly well known in fruits; but is recorded in leaves, petioles, and stems.[9] Its dehydrogenase, which cooperates with Coenzyme I, is also well known (Table 30, p. 187). Oxaloacetic acid has been reported present in pea and red clover plants;[10] but the observation could not be confirmed, even after the addition of malate and fumarate.[11] Nothing is known of plant enzymes enabling oxaloacetate to react with pyruvic acid or its oxidation products. Oxaloacetic acid is a somewhat unstable substance, which would be unlikely to accumulate to any extent, and may be liable in plants to the action of β-carboxylase which splits it to CO_2+pyruvic acid.[12] It may also be reduced to malic acid (p. 37). Plant records of the required acids and enzymes are thus at present very fragmentary, and cannot be considered to give much support or contradiction to the cyclic theory. Some of the most familiar almost certainly have other origins and significance, whether they add a respiratory one to their role or not. While the existence of many of the acids is evident, and their formation from one another possible, this by no means proves their participation in a cycle catalysing pyruvate oxidation.

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[1] Whatley, 1951*b*.

[2] Berger and Avery, 1943*a*.

[3] Barron *et al.*, 1950.

[4] See Thimann and Bonner, 1950, for other citations.

[5] Damodaran and Venkatesan, 1941.

[6] Laties, personal communication.

[7] Pucher and Vickery, 1941.

[8] Berger and Avery, 1943*a*.

[9] For a recent survey in detail see Thimann and Bonner, 1950.

[10] Virtanen *et al.*, cited from Goddard and Meeuse.

[11] Wyss, Burris, and Wilson, 1939.

[12] Vennesland and Felsher, 1946.

3. Effect on oxygen uptake

Addition of a number of the acids has been found to increase the oxygen uptake of the species investigated; but there has usually been a marked rise of the R.Q. suggesting that the acids supplied have been consumed in one way or another, rather than become members of a catalytic cycle. This is likely to happen if the acids are added in relatively large amounts, or if the tissue's mechanism is already saturated with the acid (cf. p. 234). It neither proves nor disproves the occurrence of the cycle. A single series of results with barley roots, reported by Machlis in 1944, showed 10 per cent. increases of oxygen uptake with M/20 malate, fumarate, and citrate; and 34 per cent. increase with succinate. Each time there was a small lowering of the R.Q. Spinach leaves, starved for one day or longer, showed increases within the same range with 0·1 per cent. malate, fumarate, citrate, and *iso*-citrate; and increases up to 43 per cent. with succinate. There was always a considerable rise of R.Q.[1] Oxygen uptake of oat coleoptiles is increased by dilute succinate, fumarate, *iso*-citrate, and α-ketoglutarate;[2] and that of potato slices by citrate and succinate, but not by α-ketoglutarate. Consumption of citrate and α-ketoglutarate has been observed with the latter.[3]

The effect of malate, citrate, and succinate on beetroot disks has been examined in more detail.[4] M/20 solutions caused rapid increases of O_2-consumption and a simultaneous rise of R.Q. from about 1 to 1·5 or 2. The acids supplied were consumed; and the additional gas exchange, far from being catalysed by the acids, was not even stoichiometrically equivalent to the acid disappearing. The ratio of malate disappearing to extra CO_2 formed was about 1, too high even for simple oxidation of the acid, whose fate remains uncertain.

Addition of succinate to carrot disks leads to various results depending on the experimental conditions.[5] The normal procedure in all such experiments has been to apply these acids at pH 4–5, where their dissociation is slight and entry may be expected to be fairly rapid.

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[1] Bonner and Wildman, 1946.

[2] Bonner, 1948.

[3] Barron *et al.*, 1950.

[4] Bennet-Clark and Bexon, 1943.

[5] Turner and Hanly, 1949.

Ionization appears to retard access and to promote additional activities at the cell surface. Increase of the oxygen uptake of carrot disks by M/20 succinate is maximal at about pH 4–5; and is not further raised by increasing the succinate concentration. Seasonal and varietal differences occur. The suggestive observation was made that after careful washing away of the external succinate, the respiration declines heavily; but not quite to the original level; there usually appears to be a small permanent increase of rate. It was, however, found that the effect had characteristics akin to the 'salt respiration' induced by KCl, and the authors attribute it to a similar respiration, due to the succinate ions (about half the total succinate) still present at pH 4. They consequently regard the additional oxygen uptake at pH 4 as composite, being due partly to 'the salt effect' and partly to consumption of succinate as a metabolite. It is conceivable that the persistent so-called salt effect of succinate ions depends upon their increasing catalytic activity by fortifying an acid cycle. The R.Q. of the persistent increase was about 1, but that of the temporary 'metabolic' increase was about 4. The nature of the reactions induced, as in the similar beetroot experiments, remains undetermined.

While the foregoing results may be said to demonstrate the consumption of added citrate, malate, and succinate by a number of plant tissues, there is very little in them to indicate their use to reinforce a catalytic respiratory cycle.

4. Malonate inhibition

This has been observed with a number of plant tissues in mildly acid solutions (Table 42) and may be almost total at 0·05 M. Even at low pH, in which the entry of the inhibitor is probably fastest, inhibition takes an hour or more to develop. Oat coleoptiles, starved by a period of separation from their endosperms, show much stronger inhibition than those not starved. This might result from a lowering of the internal succinate concentration, since the inhibition is competitive; but Bonner, using sheep's heart succinoxidase, found no lowering of the succinic acid content due to depletion, a result that casts some doubt on the specificity of malonate poisoning.

At pH 6, or higher, malonate inhibition disappears, and there may even be some temporary stimulation of oxygen consumption.[1] Some negative results are likely to be due to this cause. An interesting exception is provided by *Arum* spadix which is not inhibited at pH 3·5 (Table 42), and is not stimulated by succinate at the same acidity.

[1] Bonner and Wildman, 1946; Bonner, 1948; Turner and Hanly, 1947; Laties, 1949*a*.

TABLE 42

Inhibition of O_2-uptake of plant tissues by mildly acid malonate

Tissue	*Malonate concentration*	*pH*	*Inhibition*	*Reversal by*	
Barley roots	0·01 M	4·7–5·7	40%	see text	Machlis, 1944*a*
	0·05 M	4·7–5·7	90%		,, ,,
	0·01 M	4·0	65%		Laties, 1949*b*
	0·05 M	4·5	80%	pyruvate succinate fumarate α-ketoglutarate malate citrate *cis*-aconitate	,, 1949*a*
Carrot disks	0·05 M	4·0	77%	. .	Turner and Hanly, 1947
Beet disks .	. .	4·0	high	. .	Turner and Hanly, 1947
Spinach leaf	0·01 M	4·5	47%	pyruvate succinate	Bonner and Wildman, 1946
	0·05 M	4·5	93%	fumarate malate *iso*-citrate	Bonner and Wildman, 1946
Avena coleoptile	0·05 M	4·5	93%	succinate fumarate *iso*-citrate α-ketoglutarate	Bonner, 1948
Arum spadix .	0·05 M	3·5	nil	. .	James and Beevers, 1950

Arum spadix contains no metallic oxidase (p. 227) such as cytochrome oxidase through which succinate oxidation usually proceeds.

It seems clear that malonate inhibition is common. In assessing the value of this evidence, it has to be remembered that its specificity in plant tissues has not been much investigated, and has recently been called in question.[1] In Machlis's experiments with barley roots, malonate inhibited oxygen consumption and CO_2-production equally. If it were only inhibiting succinate oxidation, continued anaerobic production of CO_2 would be expected. Addition of acids of the cycle may restore oxygen uptake. Since the inhibition is competitive, with a rather high ratio in favour of succinate, this might be achieved even with acids supposed to come before the block. A number of positive results are listed in Table 42. The renewed oxygen uptake was substantial, and frequently restored the original rate; it was considerably greater than the small increases reported on adding the same acids to unpoisoned tissues.

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[1] Turner and Hanly, 1947.

5 and 6. Succinate formation in the presence of malonate

Increased oxygen consumption might result from consumption of the acids in any of a number of ways. The accumulation of succinate as a result of their feeding to malonate-poisoned tissues is perhaps the most cogent single piece of evidence that can be adduced. It has been observed after adding fumarate to poisoned oat coleoptiles,[1] barley roots, and spinach leaves.[2] Oat coleoptiles have also given positive results with α-ketoglutarate. The results are not entirely in agreement, however, since starvation was necessary to produce the effect in oat coleoptiles, and considerably reduced it in spinach. Experiments with other acids that should lead to succinate formation have not yet come to notice.

A survey of the foregoing data yields enough evidence for it to be supposed that the acids of the tricarboxylic acid cycle occur, and are probably inter-convertible in plant tissues. It further shows that some reaction susceptible to malonate poisoning is likely to be a common stage in respiration, though it does not seem certain that this stage is necessarily the oxidation of succinic acid. There is very little evidence to support the further suggestion that the acids set up a catalytic cycle of oxidation in plant tissues, and some that would make it difficult in certain species.

While the path to pyruvic acid seems fairly well mapped out by the reactions of glycolysis, its further decomposition during plant respiration remains obscure. Neither by way of acetaldehyde nor through an acid cycle has any degree of certainty yet been achieved. As a further consequence, although we have a fair knowledge of a plant's reactions with oxygen, we still know very little about the links between oxidation and glycolysis. The two ends of the chain are grasped, but its middle links are still hidden from sight.

[1] Bonner, 1948.

[2] Laties, 1949*b*.

X

CONNEXIONS WITH OTHER PROCESSES

HEAT FORMATION AND WORK

HEAT is an end-product of respiration in some respects analogous to carbon dioxide. Its release is probably invariable, and accelerates with the rate of respiration, being evident to casual observation during active germination, as on a malting floor, or in a heap of lawn mowings. The heating of moist grain in store results from the acceleration of respiration with the rise of humidity.[1] Similarly, a rise of temperature, by accelerating respiration, increases heat output also. According to Doyer (1915), wheat seedlings at 20° C. produced 2·60 kcal per hr. per kg. grain, rising at 30° C. to 6·03 kcal, equivalent to a $Q_{10} = 2{\cdot}3$, similar to the Q_{10} of CO_2-production (p. 26).

The formation of heat is most simply and usually measured by lagging the tissue in a thermos flask and observing the temperature rise. Under such simple conditions, peas have been found to release 34·6 kcal per gm. dry weight,[2] calculated from a temperature rise of 20° C. Similar rises may be observed at the centre of a large heap of cut grass soon after mowing,[3] and in *Arum* spadices wrapped about with cotton wool.[4]

Energy is therefore lost from a respiring tissue as heat, and some attempts have been made to estimate what percentage of the total energy loss this represents. It may be first mentioned that the energy loss is not necessarily equal to the whole energy turnover of respiration, since much of the transformed energy may remain permanently in the plant, locked, for example, in new products of synthesis and growth. Energy may be lost either as heat or in performing work outside the plant itself. The loss of total energy content of a tissue over any given period is given by the decrease in its heat of combustion. This can be compared with the heat production during the same period. Rodewald[5] estimated the heat production as 93 per cent. of the heat of combustion of the sugar consumed in kohlrabi stems and as 99·2 per cent. in apples. The corresponding figure for an *Aspergillus* culture is given as 94 per cent.[6] Doyer's results for germinating wheat seedlings, comparing the fall in heats of combustion of the whole seedlings with simultaneous heat emission, vary irregularly from 95 down to 38 per cent.

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[1] Gilman and Barron, 1930; Milthorpe and Robertson, 1948*a*.

[2] Pierce, 1912.

[3] Wohl and James, 1942.

[4] Church, 1908.

[5] Rodewald, 1887, 1889.

[6] Algera, 1932.

With the exception of some of Doyer's figures, these results are consistent with the observation that plants perform only negligible amounts of work. No work other than the infinitesimal amount done in raising water of guttation can be attributed to freely germinating wheat grains, and it must be concluded that Doyer's methods were liable to large experimental errors.

No plant tissue is able to perform mechanical work on the scale shown by muscle. The displacement of earth and heavy stones by growing roots is work of such a kind; but the mechanism of its performance is very different, and only indirectly dependent on respiration. The raising of water to the tops of tall trees also involves work; but, again, it goes on at the expense of energy directly absorbed as heat from the surroundings, the latent heat of the water's vaporization, and has no direct dependence on metabolic energy transfers. The dry matter of a tree's crown, representing a heavy weight, is, of course, not lifted, but synthesized from the atmosphere *in situ*. The reasons against extending the term 'work' to cover internal changes as 'internal work', 'chemical work', &c., are given by Wohl and James (1942).

The consequences of heat production to the plant are not commonly of much importance. If a rise of temperature resulted, as in the warm-blooded animals, the effects would be very marked; but, in general, the diffuse form of plants makes them such excellent radiators that rises of temperature are imperceptible. The heating of organs with low transpiration rates, which sometimes becomes considerable during strong illumination, and may become destructive to the plant, is not due to respiration; and it would be difficult to adduce any examples in which respiratory heat production caused a significant temperature rise under normal conditions. The most likely exception is the rapid opening of massive inflorescences among the palms and aroids.

SYNTHESIS

The essential feature of biosynthesis is not merely the formation of larger or more complex molecules, but an increase in their free energy. Respiration is connected with synthesis because it may provide the energy and also the material starting-points for synthetic reactions. It is not perhaps the only provider, especially in photosynthesizing tissues, where unknown intermediates and conversions of energy operate.[1]

Respiration proceeds by way of numerous chemical steps, almost any one of which might provide an intermediate product capable of being the starting-point of a synthetic sequence. The greater the number of such steps the more numerous become the possibilities of synthesis, and of turning the free energy decrease to the purposes of synthesis and growth. Instead of the energy being lost in a violent one-stage reaction of high energy turnover, it may become 'stored' in labile intermediates.

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[1] See, e.g. Burström, 1941, 1943.

The most elegant and best-understood example at present is the synthesis of labile phosphate bonds, especially in the action of phosphate during the oxidation of phosphoglyceraldehyde (p. 127). The oxidation of the aldehyde group to carboxyl proceeds with a relatively large decrease of free energy as indicated in the equation

$$\begin{aligned}&\text{3-phosphoglyceraldehyde}+\text{CoI}\\&\qquad = \text{3-phosphoglyceric acid}+\text{CoI}\cdot\text{H}_2+10{,}000\text{ cal.}\end{aligned}$$[1]

In the presence of free phosphate a phosphorylation is coupled with the oxidation and, if adenosine diphosphate is absent, 1,3-diphosphoglyceric acid accumulates.[2]

$$\begin{aligned}&\text{3-phosphoglyceraldehyde}+\text{CoI}+\text{H}_3\text{PO}_4\\&\qquad = \text{1,3-diphosphoglyceric acid}+\text{CoI}\cdot\text{H}_2+400\text{ cal.}\end{aligned}$$[3]

The energy lost in the first reaction is retained in the second with the unstable carboxyphosphate bond, $\text{—COO} \sim \text{H}_2\text{PO}_3$, of the diphosphoglyceric acid. When adenosine diphosphate is present, the phosphate and the extra energy are together transferred to generate energy-rich adenosine triphosphate; a similar formation of adenosine triphosphate occurs in the dephosphorylation of *enol*phosphopyruvic acid (p. 130). The reaction

$$\text{2-phosphoglyceric acid} \rightarrow \text{pyruvic acid}+\text{H}_3\text{PO}_4$$

has $-\Delta F$ about 16,000 cal[4] almost all of which depends upon the $\text{C:CO} \sim \text{H}_2\text{PO}_3$ group of *enol*phosphopyruvic acid, and may in large part (11,000 cal) be transferred to adenosine triphosphate.

The anaerobic breakdown of hexose yields two triosephosphate molecules, and hence may lead to the generation of 4 energy-rich phosphate bonds. One or two are consumed in the formation of hexosediphosphate leaving a net synthesis of at least two adenosine triphosphate molecules with about 22,000 cal of stored energy available for synthesis.

There is no information about the number of active phosphate bonds generated during the aerobic respiration of plants. Estimates have been made for animal tissues varying from 1 to 4 molecules of ATP formed for each atom of oxygen consumed.[5] According to Lipmann, the most probable number is 3, which would give a yield of $36-2 = 34$

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[1] Meyerhof and Junowicz-Kocholaty, 1943.

[2] Warburg and Christian, 1939; Negelein and Bromel, 1939.

[3] Ochoa, 1946.

[4] Street, 1950.

[5] Summaries by Baldwin, 1952; Street, 1950.

for each molecule of hexose decomposed. Assuming each $\sim$ P link adds = 11,000 cal, this represents a fixation of 374,000 cal or about 55 per cent. of that given up by the hexose. Various suggestions, usually involving the Krebs cycle reactions, have been made to explain how this is done. Among plants the oxidation of α-ketoglutarate by preparations from *Phaseolus aureus* has been coupled with the synthesis of adenosine triphosphate and the formation of hexose phosphate.[1]

The consequences to synthesis of the formation of the energy-rich phosphates are numerous. They lead directly to the formation of starch and glycogen, as already mentioned on pp. 111 and 116, and, at least in some organisms, to sucrose (p. 114). It is possible, though as yet unproven for plants, that synthesis involving non-carbohydrate constituents work by similar mechanisms.

In general, syntheses implying an increase in free energy occur in non-green tissues only by relation to respiration, and must be coupled to stages occurring with sufficient free energy loss. Connexion between two chemical processes is established by one or more molecules common to both of them; such as the 3-phosphoglyceraldehyde above. The increase of free energy in the resulting adenosine triphosphate is brought about only with a greater loss in the oxidation to phosphoglycerate. The synthesis may be regarded as a driven reaction and the oxidation as driving it. The system is, however, a unity and cannot be dissected without loss of its properties.

Chlorella, kept in the dark and supplied with sugar, reduces nitrate which can be used in lieu of free oxygen.[2] After an initial period in which NH_3 is used in protein formation, ammonia is released together with CO_2. In the acid media used, the overall changes could be represented by

$$NO_3' + 2H^+ + \tfrac{1}{3}C_6H_{12}O_6 \rightarrow NH_4^+ + 2O_2 + H_2O + 165 \text{ kcal}$$

a spontaneous reaction with a large decrease of free energy. In the absence of sugar, the reduction

$$NO_3' + 2H^+ + H_2O \rightarrow NH_4^+ + 2O_2 - 64 \text{ kcal}$$

would be highly endergonic and incapable of spontaneous occurrence. When the oxygen is enabled to oxidize carbohydrate,

$$2O_2 + \tfrac{1}{3}C_6H_{12}O_6 \rightarrow 2CO_2 + 2H_2O + 230 \text{ kcal}$$

provides the necessary driving reaction with an energetic efficiency of $64 \times 100/230 = 28$ per cent. Although the reduction, a synthesis in the sense of metabolic biology, may thus be regarded as a reaction driven by respiration, nothing more is known of the mechanism by which the above two reactions are joined. Although oxygen appears in both equations, it is not a direct link between them.

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[1] Millerd *et al.*, 1951.

[2] Warburg and Negelein, 1920.

A rather more fully explained linkage, connecting the synthesis of malic acid with the oxidation of Coenzyme II, has already been described on p. 37.

PROTEIN LEVEL

Tissues containing a high proportion of protein in general show fast respiration rates. This is particularly obvious in seedlings and meristems; but is demonstrably of wider application. The correlation between protein content, expressed as protein-N/dry weight, and CO_2-emission rate has been examined principally in the leaves of barley[1] and grasses,[2] subjected to a wide range of cultural treatments. Richards summed up by saying 'under normal treatment, or treatments, involving only slight deficiencies, the respiration rates of young mature leaves taken directly from the barley plant in day-time are dependent almost entirely on some aspects of their nitrogen metabolism, and are nearly independent of their carbohydrate level'. In extreme phosphorus and potassium deficiencies, sugar concentration limited the respiration rate; but in more normal conditions they would, as indicated by the data of Chapter III, be present in too great an excess to do so.

The observation that respiration rate may sometimes be correlated with the proportion of protein-N in tissues may be due to the catalytic activity of the enzymatic proteins (p. 19), but a converse relation also needs to be considered. The proteins of a tissue exist far in excess of their natural equilibrium with the amino-acids, &c., of the tissue's soluble nitrogen fraction. It may be supposed that the large increase of free energy necessary for their formation, and perhaps for the maintenance of their high concentration against a continuous degradation, is supplied by respiration; so that the rate of respiration might, though not necessarily, determine the steady state and the level at which protein concentration is maintained.

This point of view has been most fully developed by the school of Gregory, who lays particular stress on the behaviour of acutely potassium-starved barley leaves. In this condition, instability of proteins becomes strongly marked, though the proportion of protein-N does not at first fall. The amount of free amino-acid present is much increased, and the respiration rate is high when quantities are expressed per unit dry weight. This situation, the authors suggest, is most simply explained by postulating a cycle of protein formation and breakdown in which the two processes are distinct, both becoming much accelerated in potassium starvation. Amino-acids formed in the breakdown give rise by deamination to ammonia, which is utilized in conjunction with respiratory intermediates to form new protein, and to carbon residues which are further degraded to CO_2. In this way the rate of CO_2-emission may depend on the rate at which the protein cycle is traversed. Even when some CO_2 is released by direct breakdown of carbohydrates, in excess of the amount involved in protein synthesis, it may remain true that 'a given rate of CO_2-evolution can maintain only a definite quantity of protein'.

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[1] Gregory and Sen, 1937; Richards, 1938; Gregory, 1937.

[2] Petrie and Williams, 1938; Wood, 1941, 1942, 1945.

The conception of independent synthetic and breakdown processes is in line with more recent indications that the synthesis of peptide linkages may proceed through phosphorylated intermediates, both intermediates and phosphate bond energy being provided by respiration.[1] More doubtful appears to be the assumption that proteins suffer continuous breakdown in all tissues; evidence against such an assumption for vine and young cherry-laurel leaves appears rather strong (Chapter 3).

The correlations quoted have all been obtained from leaves, and it appears rather doubtful whether the energy and material necessary for their accomplishment of protein synthesis are, in fact, always provided by respiration. Attempts to induce protein synthesis in cut and darkened leaves have never led to any impressive increase of protein content. Cut daffodil leaves showed increases up to 30 per cent. of their protein-N in their growing zone when illuminated; but only slight losses in the dark.[2] The experiments of Burström (1943) with wheat leaves have provided additional information. In the absence of light, no nitrate was assimilated, the amount fixed increasing as light intensity was raised. At the same time, the discrepancy between the net CO_2-uptake and the increase of sugars in the leaves rose in proportion to the nitrate assimilation. Both effects were reduced by lowering the CO_2-content of the air. The difference between CO_2 absorbed and sugar formed was taken to give a measure of the carbon in the 'CN assimilates', which were found to have a C/N ratio $= 14$; too high to indicate protein formation alone ($C/N =$ about 4). The suggestion was therefore made that carbon compounds, such as plant acids without nitrogen, were being formed as well as proteins. Since nitrate assimilation did not occur in the dark, but only simultaneously with CO_2-assimilation in light, it could not be promoted by respiration, but must be a direct photosynthesis. This view aligns with the recent observations in photosynthesizing *Chlorella* that part of the ^{14}C of labelled CO_2 appears within 30 sec. in such nitrogen compounds as alanine and aspartic acid.[3]

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[1] Summary in McKee, 1949.

[2] Pearsall and Billimoria, 1939; Pearsall, 1949.

[3] Benson and Calvin, 1950.

It therefore appears that there are at least two different methods of protein synthesis in plants, and, whereas the dark synthesis in roots is doubtless coupled with respiration, the light synthesis characteristic of leaves cannot be assumed to be so.

PHOTOSYNTHESIS

Possible interactions with respiration have been the bugbear of photosynthetic measurements since their beginning. The usual assumption, which has given the most consistent results, is that respiration rates continue unaltered during a period of illumination. Nevertheless, evidence has been brought forward indicating that they may sometimes increase and sometimes slow down.

Arguments for an increased light respiration usually rest on observation of enhanced respiration rates immediately after removal of the light; the increase usually dying away fairly soon. Such increases have been found with a variety of water angiosperms and algae.[1] The existence of the response in *Fontinalis antipyretica*[2] could not be confirmed by the present writer at moderate light intensities, and was not found by McAlister (1937) in wheat. Spoehr and McGee (1923) found virtually no increase in sunflower leaves; but a 195 per cent. rise in bean leaves that had been depleted of carbohydrates by 64 hours' starvation. The suggestion might thus be made that the increased respiration results from accumulation of photosynthetic end-products or intermediates. This is unlikely to be the whole of the story because starved cherry-laurel leaves, illuminated in CO_2-free air, also showed the rise on being returned to darkness.[3] Further, the respiratory increase is more limited than is photosynthesis to ultra-violet and other short wave-lengths.[4] It is claimed to exist in some non-chlorophyllous tissues at wave-lengths below 500 mμ,[5] but is not shown by barley roots in the light of 500-watt filament lamps.[6] It is also favoured by extreme conditions such as high oxygen concentration[7] or intense illumination,[8] and has been attributed largely to photo-oxidations outside the normal mechanisms of photosynthesis and respiration.[9] Reduction, or even total suppression of respiration in illuminated algae has been argued by Kok (1951) on the basis of experiments at low light intensities. He considers his data to show that the relation between oxygen exchange and light intensity is not a single curve, but two curves intersecting at the compensation point. The lower curve is taken to be twice as steep as the upper curve above the compensation point, and is explained by supposing that respiration and photosynthesis are both suppressed by a third process having double the light efficiency of normal photosynthesis. 'Definite solution of this problem', he says, 'can only be expected from tracer work.'

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[1] Föckler, 1938; Gessner, 1937, 1939; van der Paauw, 1932.

[2] Föckler, 1938; Bode, 1940.

[3] Audus, 1947.

[4] Gessner, 1939; Föckler, 1938; Emerson and Lewis, 1943 (*Chlorella*).

[5] Föckler, 1938; Montford and Föckler, 1938.

[6] James and Kilbey, unpublished.

[7] McAlister and Myers, 1940.

[8] Myers and Burr, 1940.

[9] Franck and French, 1941.

To a limited extent such information is now available.[1] Air enriched with ^{18}O was used as a source of oxygen for respiration. Respiratory consumption could therefore be followed during the simultaneous production of normal ^{16}O by illuminated cells. Experiments with *Chlorella*, though not necessarily of the same strain as Kok's, showed no alteration, due to light, in the rate of oxygen uptake. There was also no effect with species of *Pinus*, *Nicotiana*, and *Metasequoia*, or with barley and spinach. Some other algae, *Chroococcus*, *Porphyra umbilicus*, *Ulva lactuca*, and *Anabaena*, showed marked reduction of oxygen uptake especially at high light intensities. No increases of oxygen uptake were observed.

The negative results with *Chlorella* might be thought surprising because of the high quantum efficiency of its photosynthesis at low light intensities, and the current suggestion that respiratory intermediates, particularly phosphoglycerate, phosphoglyceraldehyde, and pyruvic acid also arise by photosynthesis.[2] Both these observations could indicate a close connexion between the two processes. The lack of any light effect on oxygen uptake cannot be taken to disprove such a connexion, since photosynthetic centres are localized in plastids, and respiratory centres appear to be well distributed. The number of respiratory centres near enough to the sites of photosynthesis to be immediately affected by their local accumulation of intermediates might well be too small to affect the gross respiration rate.

Transfer of respiratory intermediates and energy to assist photosynthesis, particularly in weak light, has been much debated. The respiration of *Hormidium*[3] and *Chlorella*[4] is considerably increased by supplying glucose; but there is no corresponding increase of photosynthesis. The additional glucose respiration of *Chlorella* is inhibited by cyanide, whereas its autonomous respiration survives; so it is possible to argue that the normal respiration could be linked to photosynthesis even though the additional respiration was not. Most of the arguments have rested on determinations of quantum efficiency[5] and, as Franck puts it: 'There is, however, no sense in discussing here these theoretical considerations as long as the interpretations of the experimental results . . . are still so divergent.'

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[1] Brown, Nier, and van Norman reported by Gaffron and Fager, 1951.

[2] Calvin and Benson, 1948; Gaffron and Fager, 1951.

[3] van der Paauw, 1932.

[4] Emerson, 1927.

[5] Kok, 1951; Franck, 1949, 1951.

Of the many possible contacts between photosynthesis and respiration, the only ones yet proved to exist are by way of end products: carbon dioxide, oxygen, and sugars.

SALT UPTAKE

Up to about 1930 it was supposed that the principal source of the energy driving dissolved substances into plant cells was the thermal energy of the diffusing ions or molecules themselves. It was known[1] that the average concentration inside the tissue might come to exceed the external concentration considerably; but this was attributed to an establishment of adsorption or Donnan equilibria depending on the presence of immobilized protein and other ions in the cytoplasm. The importance attached to adsorption diminished when the vacuolar sap was extracted from the relatively large coenocytes of *Valonia*, *Nitella*, and *Halicystis*.[2] It was then discovered that *Nitella* sap contained much higher concentrations of sodium, potassium, calcium, magnesium, and chloride than the surrounding pond water, and that *Valonia* concentrated free potassium ions from the sea.[2] Similar observations were made with *Halicystis*. Conductivity measurements on *Nitella* sap showed that the ions were in free solution, not adsorbed or precipitated; and the increased ionic product was apparently not explicable as a Donnan equilibrium. The same probably applies to the higher plants, and an examination of decapitated barley roots has shown that sap exuding from the cut surfaces, or sap obtained by rapid freezing of the tissues, may have accumulated potassium and bromide ions in great excess over their concentration in the solution bathing the roots.[3] This accumulation may be much more pronounced than accumulation in the relatively slow-growing algal coenocytes.

Salt uptake against concentration or activity gradients is indeed characteristic of rapidly growing and metabolizing tissues such as meristems, and is not observed in the mature and senescent.[4] It reveals a connexion with respiration in requiring oxygen and sugar, in having a high temperature coefficient, and in being reduced by respiratory inhibitors. Although it is possible to inhibit salt uptake without stopping respiration, no inhibitor is yet known to stop respiration without stopping salt accumulation. Tissues that have been long washed in water, when they are transferred to salt solutions, may show an increased rate of respiration which dies away again on return to water.

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[1] Stiles, 1924.

[2] Cooper and Blinks, 1928; Hoagland, 1944.

[3] Hoagland and Broyer, 1942.

[4] Steward, 1935; Berry and Steward, 1934; Helder, 1951.

When the fleshy storage tissues of potatoes are sliced, the newly exposed cells show a regeneration, which may lead to new cell formation, and which is accompanied by rapid respiration and disappearance of starch. There may at the same time be rapid uptake of salts, and it was first shown by Steward that this uptake is conditioned by the concentration of oxygen.[1] At low oxygen concentrations, the uptake of both ions of KBr was reduced, and in the absence of oxygen became almost nil. The necessity for oxygen in the active uptake of salts has since been noted in barley roots,[2] *Elodea*,[3] *Vallisneria*,[4] and wheat.[5] The absorption of asparagine and other ionized organic compounds into *Drosera* tentacles and submerged *Vallisneria* leaves needs it also.[6] The rate of salt uptake declines more or less in parallel with the decline of oxygen consumption, and shows no relation with the rate of anaerobic CO_2-production as oxygen concentration is lowered.

Barley roots aerated for a considerable period in water or calcium sulphate solution have a low sugar content and a low capacity for salt uptake. Subsequent rapid uptake of potassium salts can be induced by adding sugar to the solution.[7] No effect of sugar on the uptake of K^+ ions was observed with maize, though the uptake of phosphate ions appeared to be accelerated.[8] Respiratory consumption of sugar by barley roots reaches its maximum in about 20 per cent. oxygen, and uptake of potassium, halide, and nitrate ions was observed to reach a similar peak. Both declined as oxygen concentration was lowered, but sugar consumption continued in the absence of oxygen on account of anaerobic respiration, whereas salt uptake came to a stop.[9]

High temperature coefficients for active uptake have been observed in barley roots,[10] *Nitella*,[11] potato and artichoke disks,[12] and *Elodea*.[3] Usually the uptake of both ions is similarly affected, though this may not happen always.[13] According to Hoagland and Broyer, the Q_{10} of salt accumulation in excised barley roots, held in the range 6–24° C., is above 2, and may rise to 5.

Salt uptake has been shown to be reduced by numerous respiratory inhibitors. The most frequently used has been M/1,000 cyanide.[14] It is claimed that the respiration of wheat roots is greatly reduced; but that a relatively small cyanide-resistant respiration remains. The uptake of chloride ions is more sensitive, and is extinguished at this concentration. The results are taken to indicate that there is a cyanide-resistant ground respiration that cannot be linked to salt uptake; and a cyanide-inhibited fraction that can. As cyanide concentration increases, the sensitive fraction of respiration and the salt uptake decline in parallel. Carrot disks that have been long washed in distilled water exhibit a slow respiration that is unaffected by dilute cyanide. When simple salts are added to the water a faster rate of respiration develops, and the additional 'salt respiration' is totally inhibited by M/1,000 cyanide. This inhibits all metallic oxidases to a considerable degree. When more specific inhibitors are used, it is found that the inhibitor appropriate to the tissue oxidase also inhibits salt uptake. Thus the salt respiration of carrot disks has been shown to depend to a very large extent on cytochrome oxidase (p. 220), and both the respiration and salt uptake are partially inhibited by carbon monoxide, the inhibition being reversible by light.[1] Similar results have been obtained with wheat roots.[2] Conversely, the root tips of 11-day barley seedlings afford a tissue whose respiration depends on a copper system (p. 223) and not on cytochromes. Their uptake of labelled Rb^+, Br^-, and phosphate ions,[3] is strongly inhibited by M/1,000 dieca (sodium diethyldithiocarbamate), which has little effect on cytochrome oxidase, but inhibits ascorbic oxidase totally, and the respiration of barley root tips up to 66 per cent.[3]

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[1] Steward, 1933; Steward, Berry, and Broyer, 1936 (artichokes and carrots).

[2] Hoagland and Broyer, 1936, 1942.

[3] Rosenfels, 1935.

[4] Arisz, 1947*a* and *b*, 1948.

[5] Lundegårdh and Burström, 1935; Lundegårdh, 1945.

[6] Arisz, 1942*a* and *b*, 1943; Arisz and Oudman, 1938.

[7] Hoagland, 1944; Hoagland and Broyer, 1936.

[8] van Andel, Arisz, and Helder, 1950.

[9] Hoagland and Broyer, 1936.

[10] Hoagland and Broyer, 1936; Hoagland, 1944.

[11] Hoagland, Hibbard, and Davis, 1926.

[12] Steward, 1937.

[13] Wanner, 1948.

[14] Lundegårdh, 1949 (wheat roots); Machlis, 1944; Milthorpe and Robertson, 1948 (barley roots); Robertson and Turner, 1945 (carrot disks).

The carbon monoxide results have been used to support the theory that the uptake of anions is directly linked to the transport of electrons through the cytochrome system.[4] The system is not, however, universal and uptake of Rb^+ and Br^- by 10-day barley roots with no cytochrome system is inhibited by carbon monoxide though their respiration is not.[3] In considering such data, it is necessary to remember that other respiratory inhibitors of quite different type will also affect salt uptake. Machlis (1944*a*) showed that the uptake of Br^- into barley roots was strongly inhibited by malonate and iodoacetate and was almost stopped by the latter at 10^{-4}M. This is particularly informative because iodoacetate affects anaerobic stages of respiration (p. 218) which cannot be directly linked with salt uptake. It can only inhibit accumulation of salts because, in stopping an anaerobic stage, it slows down and finally stops an aerobic one. The same considerations might apply to the oxidase inhibitors, which are likely to stop the flow of electrons along several steps, not only in the one directly affected. Experiments with highly selective inhibitors, the loci of whose effects are known, strengthen

[1] Weeks and Robertson, 1950.

[2] Sutter, 1950.

[3] James and Russell, unpublished.

[4] Lundegårdh, 1950.

the evidence of connexion between salt accumulation and respiration, but cannot by themselves identify the connecting link.

The inhibitor evidence also needs scrutiny in the light of the fact that some substances which accelerate respiration at low concentrations simultaneously reduce salt uptake. Sodium azide sometimes inhibits respiration and sometimes increases it;[1] but so far as is known never accelerates salt accumulation in cells and inhibits strongly at M/1,000.[2] Beech roots with mycorrhiza[3] may show simultaneous acceleration of oxygen uptake and complete inhibition of phosphate absorption in the presence of dilute azide. The increased oxygen uptake is accompanied by an even larger CO_2-output, and the same results are given by roots deprived of their mycorrhizal sheaths.[3]

Somewhat similar results are well marked with 2,4-dinitrophenol in concentrations around 1 in 100,000. Acceleration of oxygen consumption has been observed in oat coleoptiles,[4] tobacco callus tissue,[5] and carrot disks.[6] Uptake of KCl by carrot disks is completely inhibited at the same concentration. It is evident that respiration can be uncoupled and made ineffective in salt uptake, and probably in more ways than one. The extra respiration in the presence of azide is unlikely to depend on a metallic oxidase, whereas the extra respiration of carrot disks induced by dinitrophenol was sensitive to carbon-monoxide inhibition and apparently mediated by cytochrome oxidase.

Acceleration of oxygen consumption may result from moderate increases of internal permeability, as in the presence of traces of chloroform or ethylene, and such changes might be expected to reduce the retention of accumulated salts. There is at present no direct evidence that either azide or dinitrophenol affect permeability in low concentrations, and dinitrophenol caused only slight leaching from carrot disks, at far too slow a rate to account for the stoppage of accumulation. On the other hand, there is reason to believe that dinitrophenol stops the transfer of energy-rich phosphate,[7] and the suggestion arises that phosphate-bond energy is, in some way at present unknown, used to accumulate salts against their concentration gradients.

There is a certain amount of evidence that entry of ions into the protoplasm and thence into the vacuole are two distinct processes.[8] The observations of Overstreet and others[9] on salt uptake by barley roots at low temperatures are of special interest. Owing to its high temperature coefficient, active salt uptake can be partially eliminated by bringing the temperature near to zero. The use of radioactive isotopes of rubidium and potassium showed that exchange of labelled ions with unlabelled ones continued to go on at these low temperatures. Phosphate ions were also taken up; but few or none escaped. The uptake by these means was most obvious at the apex of the root; and losses of potassium from salt-rich roots were little affected by changes of temperature. Interchange of potassium ions went on until 10 per cent. of the total potassium content had been exchanged, and it was suggested that this exchangeable potassium was associated with the colloids of the protoplasm and cell wall. It seems reasonable to suppose that the remaining non-exchangeable potassium was present in the vacuole, and this would be in line with the observation of Brooks[1] that radioactive Rb^+ ions penetrated rapidly into the protoplasm of *Nitella*, where they were recorded within a few minutes; but did not arrive in the vacuole in sufficient quantities for detection in the first 6 hours. Calcium ions are also readily exchanged between the protoplasm of *Elodea* cells and the external solution; but no calcium is found in the vacuole.[2]

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[1] Marsh and Goddard, 1939*a*; Stenlid, 1949*a*.

[2] Stenlid, 1949*a*; Russell, unpublished (barley roots).

[3] Harley and McCready, unpublished.

[4] Bonner, 1949*a* and *b*.

[5] Newcomb, 1950.

[6] Robertson, Wilkins, and Weeks, 1951.

[7] Loomis and Lipmann, 1948; Teply, 1949.

[8] For general discussion see Arisz, 1945.

[9] Overstreet and Broyer, 1940; Broyer and Overstreet, 1940; Overstreet and Jacobson, 1946.

Van den Honert (1933) made an extensive series of measurements of the rate of uptake of phosphate into sugar-cane roots. He found the relation between rate of uptake of the $H_2PO_4^-$ ion and the phosphate concentration in the external solution was approximately logarithmic, and akin to the Freundlich isotherm for an adsorption equilibrium. The chief departure from the logarithmic relationship, a flattening of the curve as saturating concentrations were approached, may also be found in adsorption systems. He took this to indicate that the first step in the uptake was adsorption to a protoplasmic colloid.

It thus seems possible to suppose that ions enter the protoplasm, either by free diffusion or by exchange with other ions already there. A limit may be set to this entry by the tonoplast or other membrane within the protoplasm, which offers high resistance to the passage of ions. It appears to be the more difficult passage of this membrane that requires oxygen and active metabolic help for its occurrence.

The energy turnover of the respiration associated with salt uptake greatly exceeds the amount needed to overcome the concentration gradient. The additional respiration induced in well-washed carrot slices by the presence of 0·01 M potassium chloride may be considerable and equal to the 'ground respiration'. It has been calculated that the ratio of the additional energy turnover to the energy required to be expended in the simultaneous salt uptake may be as high as a hundred.[3] It is therefore possible to regard salt accumulation as a process driven by respiratory energy providing that a suitable mechanism exists, i.e. if the entering substances react with respiratory intermediates or products under appropriate conditions. One obvious condition is that the respiratory component is placed under a restraint, preventing it from escaping from the reaction zone of the cell. The failure of either metabolites or enzymes to escape from healthy cells immersed in non-toxic solutions is a familiar fact, though the reason for it is still uncertain. In some cells the restraint may take the form of a surface membrane, but it may also be a general property of the proteins of the cytoplasm (p. 11), anchoring some molecular species, but allowing free movement to others. The essential is reaction between a molecule or ion free to move and one that is unable to escape into the external solution. Secretion into the vacuole occurs when the compound so formed migrates either into the vacuole or at least to its boundary surface, and there breaks down with release of the original entrant, and reconstitution of the respiratory product in its original or some other form. The vacuole boundary is supposed permeable to the compound, but impermeable to the accumulated entrant. It is conceivable that reactions might occur between respiratory intermediates and non-ionizing substances entering the cell; but Arisz (1942) found that the uptake of ionized organic compounds, such as asparagine, into *Drosera* tentacles was much more affected by the presence of oxygen than was the uptake of unionized or slightly ionized compounds.

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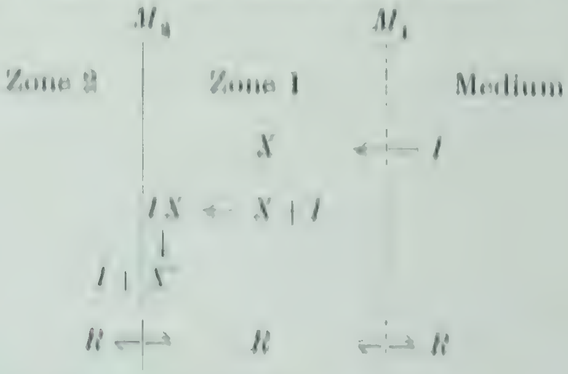
[1] Brooks, 1940.

[2] Mazia, 1938.

[3] Robertson, 1941.

When ionization occurs one ion may be supposed to react with the respiratory component, while the entry of the other is but little opposed by the resistant membrane, and accelerated directly or indirectly by electrostatic forces. Alternatively it might prove necessary to assume that two respiratory components are produced, one reacting with cations and the other with anions.[5] Since competition occurs between ions of like sign, it is not necessary to assume a specific binding compound for every ion. The system may be generalized as follows:[6]



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[5] Jacobson, Overstreet, King, and Handley, 1950.
[6] After Wohl and James, 1942, slightly modified.

Zone 1 is the site of respiratory reactions leading to the formation of component X, which is unable to escape, M_1 representing the constraint, whether by means of a surface membrane or otherwise. The substance I enters zone 1 and reacting with X forms IX capable of migrating into zone 2. Breakdown of IX liberates I, unable to escape by reason of the constraint at M_2, and X' which may be identical with X or differ from it. R is a second external substance, such as an oppositely charged ion, capable of more or less free entry. This generalized scheme could be applied to an absorbing vacuolated cell, in which zone 1 is equivalent to the peripheral cytoplasm and zone 2 to the vacuole; or, at a higher morphological level, it could be applied to a secretory tissue such as a nectary or perhaps to circumvasal parenchyma. It might be appropriate to the entry of phosphate ions capable of esterification with cell carriers, or $-NH_4^+$ ions forming amino or amido groups. In such examples the distinction between uptake and assimilation almost vanishes. It is not quite so easy to see how such ions as K^+ and Cl^- or Br^- can be bound upon carriers to give compounds of low dissociation. It is, however, claimed that K^+-binding compounds have been found in the bacterium *Escherichia coli*.[1] The compounds existed only during active metabolism, and disintegrated during its absence or attempts to extract them. After addition of glucose, fixation of potassium was rapid and considerable. It was suggested that potassium forms salts with hexose or other phosphate esters, built by phosphate transfers from ATP. Rubidium behaved similarly to potassium and competed with it; but sodium was not fixed. Similar attempts upon root materials seem much to be desired.

In the present position of affairs, it seems highly probable that accumulation of salts in actively metabolizing plant tissues depends upon respiration both for the energy necessary to move them against their concentration gradients, and for the continuous production of a carrier by which they are moved across a 'membrane' impermeable to their free ions. Concerning the identity of the carrier and the means by which the energy transfer is achieved, virtually nothing is yet certain.

CONCLUSION: THE SIGNIFICANCE OF RESPIRATION

The simultaneous occurrence of life and respiration has been the subject of frequent remark; and so far as active life and active respiration are concerned appears to have no exceptions. The conclusion has been drawn that, since life never occurs without respiration, respiration must be essential to it. The same type of argument has not been so popular as a proof, for example, that the human young, never lacking a vermiform appendix, could not be safely born without it; or that pillar boxes could not perform their postal functions upon ceasing to be red. There does not appear to be any *a priori* reason why plant respiration should not prove to be, for example, an evolutionary vestige or mere irrelevance.

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[1] Cowie, Roberts, and Roberts, 1949; Roberts, Roberts, and Cowie, 1949.

Most pronouncements upon this subject are like the examination candidate's remark, 'respiration liberates energy for the life of the plant' in having a strongly teleological savour. They may, within their province, be correct; but their placid acceptance would do little to advance our knowledge of the process, and much to stifle further inquiry; in Bacon's formulation: 'Not because those final causes are not true and worthy to be enquired, being kept within their own province; but because their excursions into the limits of physical causes hath bred a vastness and solitude in that tract.'[1]

In highly adapted organisms, most aspects of their structure and working may be considered to have some value to the organisms themselves. In particular, their metabolism may be closely adjusted to what is best for survival; and it becomes, in consequence, possible to discuss their metabolic reactions in relation to more or less clearly defined ends.[2] Nevertheless, it is well to hold in mind that the ends are presumed or guessed, not revealed.

The position is entirely different with primitive organisms, whose degree of adaptation is much less. It would be difficult to contend that the formation of alcohol was in itself of advantage to yeast; or that the toxins, inconvenient to the host, were advantageous to the pathogens producing them, and which they, in fact, frequently destroy. It is true that the formation of these end-products is associated with reactions that appear to be essential and cannot, by the organisms in question, be divorced from them. The significant fact would seem to be that essential reactions may carry with them lethal as well as vital consequences, and that the mere invariable occurrence of a process is no guarantee of its value. This is particularly conspicuous among the bacteria with their protean metabolism, and in Stevenson's words: 'it now seems clear that a belief in the functional importance of all enzymes found in bacteria is only possible to those richly endowed with Faith'.

The higher plants may be regarded as taking a position somewhere between the two extremes of the bacteria and mammals. Better, or at least more rigidly, adapted to their surroundings than the bacteria, their tissues still lack the extreme specialization of those of the higher animals. They produce an immense range of metabolic products, such as alkaloids and polyphenols, which cannot be shown to have any particular importance to their producers; and it seems little better than an even chance that a metabolic reaction occurring in a plant tissue will be of any real importance to it.[1]

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[1] *Advancement of Learning.*

[2] Cf. Peters, 1938.

Assuming that some part of the respiratory changes is important in promoting growth and synthesis (p. 242), the question remains whether the respiration of mature tissues, no longer growing, has any significance. Living tissues exist out of equilibrium with their surroundings, and have internal phases also out of adjustment with one another. The continuous maintenance of such disequilibria might result from spatial separations, or might only be achieved by a continuous expenditure of energy (cf. p. 13). It is an obvious possibility that the continuous turnover of energy, represented by the respiration of a mature tissue doing no work and little synthesis, might be linked to such protoplasmic maintenance. Unfortunately, there is at present no valid evidence, either for or against the idea.[2] The maintenance of the living state in a cell cannot involve more than a very small fraction of the large heat-production going on. Meyerhof (1924), on killing erythrocytes in a calorimeter by addition of acrolein, observed no measurable release of heat. The continuous destruction of carbohydrate and liberation of heat in the respiration of higher plants may be to a very large extent wastage, similar in kind—though less in degree—to the changes occurring when yeast gets into a sugar solution or a bull into a china shop. It is possible to regard metabolic evolution as an increasing efficiency in the use of material and energy; but it is not clear that from this standpoint the higher plants have evolved very far.

From a more general standpoint, plant respiration may be regarded merely as a path of energy degradation. By reducing the required energy of activation to finite proportions, a catalyst enables a reaction to proceed. The evolution of enzymes may be regarded as the appearance of more and more efficient catalysts, i.e. as the production of easier and more probable paths of energy degradation. This is true, even though complex and improbable chemical and morphological structures arise by the way. So long as there is a net loss of free energy, the new type of change goes on and augments the total degradation of energy. The end is a uniform low potential of energy and a maximum entropy. In focusing our attention as biologists upon incidental local increases of free energy associated with respiration and synthesis, we are concentrating upon temporary incidents of the energy flow, of about as much overall significance as the eddy under the bank is to the flow of the river to the sea.

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[1] James, 1950*a*.

[2] Wohl and James, 1942.

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